# PRACTICAL PHYSIOLOGY

HEMMETER

# MANUAL OF

# PRACTICAL PHYSIOLOGY

Designed for the Physiological Laboratory Course in the Curriculum of the American Association of Medical Colleges

BY

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WITH 55 ILLUSTRATIONS

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# IDEALS OF PHYSIOLOGIC ENDEAVOUR

"Ich behaupte aber dass in jeder besonderen Naturlehre nur so viel eigentliche Wissenschaft angetroffen werden könne, als darin Mathematik anzutreffen ist."—Kant, in preface to "Metaphys: Anfangsgründe der Naturwissenschaft" (Werke, Ed. Hartenstein, Vol. IV, p. 360).

"I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of meager and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely in your thoughts advanced to the stage of science."—(Kelvin.)

TO

JACQUES LOEB

IN APPRECIATION OF ENCOURAGEMENT AND INSPIRATION

RECEIVED THROUGH HIS GENIUS

THIS VOLUME IS DEDICATED BY THE AUTHOR

IN PRIMIS HOMINIS EST PROPRIA VERI
INQUISITIO ATQUE INVESTIGATIO.—*Cicero*

# PREFACE.

This little book is the outcome of the last ten years of teaching experience in the physiologic laboratory of a large medical school with a view to ascertaining two important inquiries. *First*, how much of practical physiology can be taught to medical students in the limited time alloted to this department in the scheme of instruction prescribed by the *American Association of Medical Colleges? Secondly*, in endeavoring to do the work thus circumscribed as thoroughly as possible—to what extent can justice be done to the newer advances of our science which are almost entirely in the domain of General or Comparative physiology? These two aspects, all important and indispensable as they are, represent divergent tendencies. The first is toward concentration and narrowing down of work, the second leads toward expansion.

The time for laboratory work and the mental capacity to understand, execute, and apply it are limited and therefore the principle "*teach a few things thoroughly rather than many things superficially*," would appear beyond a doubt to be correct and feasible. Yet if such a laboratory system becomes too restricted and simply confined to the mechanical execution of a few schematically arranged lessons and problems, it will inevitably acquire a lifeless rigidity, becoming a discipline "without soul." It has for the last twenty-five years been preached that the instruction in Physiology by the spoken word alone is inadequate—in fact, some extremists have even designated the lecture as an "anachronism."

The most experienced teachers now advise not only a didactic discipline but even in the laboratory—over the operating tables and apparatus—word teaching simultaneously with actual manual and mental execution of problems gives the best results. It is for this reason that our little work does not represent merely a series of

"cast iron" directions, but along with an elastic scheme it aims to give the reasons or to elicite the reasons from the students just why this or that result should occur under such given conditions. Even applications to the irregular processes are not misplaced in guiding a medical student, especially if both the regular and the irregular process can be better understood thereby. In the modern medical curriculum there is such an enormous demand upon the time and intellectual capacity of the student that we are compelled to make a selection of that which is most important. The entire domain of human physiology alone is too comprehensive for the brief periods allotted to the laboratory work. (Ars longa—vita brevis.) The most important aim of the teacher, it appears to me, should be not to demonstrate or teach a great mass of individual facts from every chapter of human physiology, not to give an abundance of disconnected memory material, which really could more readily be acquired mechanically out of text-books—but rather to inculcate a plastic picture—a deep insight into the whole biochemic and biophysic driving mechanism of the living substance. Such a picture should represent a clarified and unified complex of fundamental conceptions, on to which the special and single facts shall, as it were, crystallize of their own accord, *i.e.*, without much special effort on part of the student.

There are systems of economics of intellect and in physiologic pedagogy and physiologic discipline they demand no cast iron rigid schemes, but fundamental concepts—life-like, plastic aspects, not a mass of disconnected, single knowledge. Only that which is assimilated of and by itself continues to live and work in the intellect. Knowledge of isolated single facts acquired artifically and laboriously is either stillborn or soon atrophies.

The secret to success in this kind of teaching is to be found in General and Comparative physiology. All school physiology must eventually become more and more subservient to it and be guided by it. Without the acquisition of a certain amount of general physiologic knowledge, no deep insight into human physiology can be gained. The physician who possesses a good

training in general physiology, has a more penetrating grasp of the processes in diseased cells, for he is already familiar with basic principles that constitute the bed rock upon which the working mechanisms of all living cells of all living creatures are founded. Therefore it can truly be asserted that "*the better a physiologist a man is—the better clinician he will be.*"

Even in a guide for laboratory work these basic principles of action in the living substance cannot entirely be omitted. A number of the most comprehensive of these are brought out in the discipline on the laws of *irritability*, which for the physician are so important because all diseases are merely the external expression of deviations from the normal caused by irritations of one form or another. Next to the laws of irritability an insight into the modern concepts of *osmotic pressure* as formulated by DeVries vant' Hoff, Arrhenius, H. J. Hamburger, and Jacques Loeb offer the most fertile lines for combined laboratory and didactic teaching and mental training. Then follow the Doctrines on *fermentation*, on *cell catalysis*, on *immunity*, *internal secretion*, on the *effects of inorganic salts on life processes*, etc.

The aim has been to start the practical instructions in as simple a manner as possible and gradually introduce, explain, and apply the Doctrines of General physiology.

The apparatus used is almost entirely that of the Harvard Apparatus Company, the excellence of which American physiology ows to the genius of Dr. Wm. T. Porter. Even if this talented worker had not made those many contributions of enduring value to physiology for which he is known, our science is lastingly indebted to him for the invention and improvement of absolutely indispensable apparatus which really made practical physiology possible in a much broader manner than it was before these instruments were perfected. As we could not be expected to improve upon the language of the designer we have largely used his own words in the description of apparatus. (From his *Introduction to Physiology.*)

Time and again physiologists and biologists have endeavored to state in epigrammatic form what they conceived the province

of physiology to be and even recently we were given to understand by Verworn that physiology was "*the Chemistry of the Proteids*," while another physiologist defined physiology as *the chemistry of the enzymes*. If these expressions be correctly quoted (see Science, October 6, 1911) we can only be astonished at the overwhelming control of even great minds by enthusiasm in very special and strictly limited domains of physiology. As the clearest proof of the incorrectness of defining physiology by an epigram I need only to call to mind the enormous mass of physiologic facts, truths, and doctrines that are already established and which lie entirely outside of the territory of chemistry of proteids and enzymes. Above all this, such expressions leave no room for the eminently important rôle that physics exert in the phenomena of life. It is principally these latter phenomena—those that become more intelligible from the standpoint of the physicist that lend themselves for more effective teaching in the physiologic laboratory.

The entire practical teaching of the general physiology of nerve and muscle is to a large extent a physical problem; the much extolled "*chemistry of enzymes*" can as well be comprehended under a system that makes use of the "*physics of colloidal matter*" and whoever is interested in the directing control of the special form of physical energy known as "*surface tension*" should read the article on the greater problems of Biology by D'Arcy W. Thompson in Science, October 6, 1911, pp. 423–425.

Jacques Loebs' intensely interesting work on the "*Dynamics of Living Matter*," is to my mind as a contribution, as much to biophysics as it is to biochemistry and some of the absorbingly interesting phenomena which the genius of Jacques Loeb has brought to light (especially those of contact irritability) could be at least partially explained as manifestations of physical energies.

These aspects should induce us to guide our pupils at first at least along the better understood paths of physical laws, then along those of chemistry. We shall have more than enough to work on for many years to come, and need not trouble ourselves about mystical hypotheses that cannot be submitted to experiment.

Usually an author has to thank his associates for what has gone into his work but I have to thank Dr. Charles C. Conser and Dr. Albert H. Carroll more for what they have taken out of it, for the main object was concentration. Both have aided me also in the proof-reading and line drawings. Dr. C. C. Conser prepared the alphabetic index and table of contents.

Mr. W. G. Haines, a senior student of the department of medicine, deserves mention for the typewriting and Dr. George W. Hemmeter, Demonstrater of physiology for his aid in proof-reading.

A word of explanation is necessary regarding the absence of practical exercises on the physiology of special senses, on the balance of income and outgo of energy and matter, the specific rôle of food stuffs, nutritive needs and dietary standards, etc.

These important subjects are not here included because their demonstration involves an extensive equipment usually more likely to be found in association with the respective clinical chairs—for example, the phenomena of sound perception are more thoroughly demonstrated in the clinic on ear diseases—and the principles of nutritive needs and dietary standards and the specific rôle of food stuffs (rôle of different proteins in nutrition and growth) do not lend themselves for practical exercises in the students' laboratory curriculum. In brief all subject matters that are sufficiently demonstrated in the work presented by other chairs should in the physiologic "*practicum*" be restricted in favor of demonstration of processes that are not dwelled upon by these departments. In future additions I expect to displace such a matter as enumeration of erythrocytes by other exercises that are purely of physiologic importance.

*Omission:* Experiments on pages 178 and 179 on the control exercised by the sympathetic nervous system on the secretion of the adrenal glands are quoted from the publication of Walter B. Cannon, *Journ. A. M. A.*, March 11, 1911, page 742. Just as the final proofs of this volume went to press, W. B. Cannon and also S. J. Meltzer reported new experiments on the

effect of stimulation of the splanchnic nerves (cut distal end) on the content of adrenalin in the blood.

After extirpation of the superior cervical ganglion (see page 166) Meltzer describes dilatation of the pupil when the peripheral end of the splanchnic was stimulated which is interpreted as indicating an outpouring of epinephrin into the blood. The physiologic connection of these events is explained in the text.

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## CHAPTER I.

### MUSCLE AND NERVE.

### Experimental Stimulation of the Sartorius or Gastrocnemius Muscle and the Sciatic Nerve of the Frog.

*Apparatus.*—Pithing needle, large scissors, knives, forceps, glass seeker, frog board, glass plate, bowl, towels, normal saline solution, wires, Bunsen burner, NaCl crystals, ammonia, concentrated salt solution.

**Killing of the Frog.**—Destroy the brain of the frog by plunging a pin through the skin and soft tissues covering the space between the occipital bone and the first vertebra until the point is stopped by the vertebra. Turn the pin toward the head and push it into the brain cavity; move it from side to side to destroy the brain. Pass the pin into the spinal canal and destroy the spinal cord. With a stout pair of scissors cut off the body behind the forelimbs. Remove the viscera and the abdominal walls. The operation with the pin is called pithing.

**Skinning of the Legs.**—Grasp the stump of the vertebral column of the posterior part, with the thumb and index-finger of the left hand and with the right the loose skin lying above dorsally. Now pull the hands apart and the whole skin of the legs can be easily removed.

**Bones of the Pelvis and the Legs.**—The body of the last of the nine vertebræ of the frog is articulated with the coccyx, a long rod-like bone. The transverse processes of the same vertebra

are joined by synchondrosis to the saber-like ischia, which are bound to each other by the symphysis pubis. The symphysis sustains laterally the innominate bones.

The upper leg has a simple, tube-like bone, as also the lower one.

**Fundamental Rules for the Making of Physiological Preparations.**—Avoid stretching or pressing the organ which is to be prepared for physiological experimentation, and try to touch it as little as possible.

To prevent the drying out of the preparations, keep them moist with physiological salt solution (0.6 to 0.9 per cent.).

**Preparation of the Sartorius Muscle.**—The muscle extends from the anterior part of the symphysis pubis to the knee-joint. Grasp the tendon of the muscle at the knee-joint with the forceps in the left hand; cut with the scissors in the right hand, through the tendon below the forceps, then with the scissors cut through the fascia by which the muscle is still connected with the adjoining organs, and pull it lightly upward with the forceps. After the upper end of the muscle has been laid free in this manner, cut through the upper end of the tendon also. Place the muscle preparation on a glass plate.

## Experimental Stimulation of the Startorius Muscle.

1. *Mechanical Stimulation.*—Cut from the end of the muscle lying on the plate a small piece; during the cutting the muscle contracts.

2. *Thermal Stimulation.*—Touch the muscle with a hot wire; the muscle contracts. Immerse the preparation of the other sartorius in water which has been warmed to about 50° C. The muscle enters continuous contraction.

3. *Chemical Stimulation.*—Apply to the muscle a crystal of common salt. In a short time the muscle begins to twitch.

Apply a drop of ammonia to the muscle. The muscle passes into continuous contraction (tetanus).

**To make a Nerve-muscle Preparation.**—Hold the frog by

the hind legs, back upward; the front part of the body will hang down, making an angle with the posterior portion. With the strong scissors divide the backbone anterior to this angle and cut away all the front portion of the body, which will fall down by its own weight. Make a circular incision at the level of the tendo Achilles, and another at the lower end of the femur, through the skin. The sciatic nerve must now be dissected out as follows:

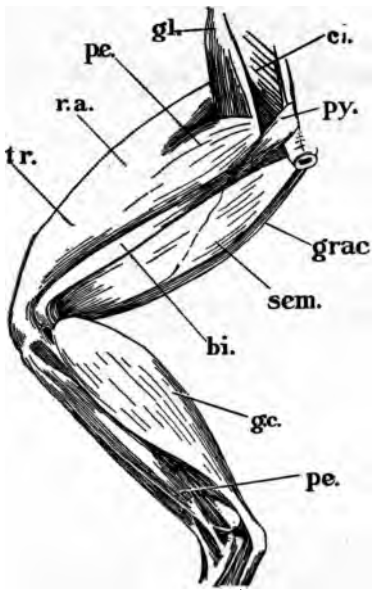



FIG. 1.—Dorsal view of frog's left hind leg. (*Enlarged from Wiedersheim and Ecker.*)

Remove the skin from the thigh, and, holding the leg in the left hand, slit up the fascia which connects the external and internal groups of muscles on the back of the thigh. Complete the separation with the glass rod. Cut through the iliac bone, making sure that the blades of the scissors are well pressed against the bone, otherwise there is danger of severing the sciatic plexus. Now divide in the middle line the part of the spinal column which

remains above the urostyle. A piece of bone is thus obtained by means of which the nerve can be manipulated without injury. Seize this piece of bone with the forceps, and carefully free the sciatic plexus and nerve from their attachments right down to the gastrocnemius muscle, taking care not to drag upon the nerve. The muscles of the thigh will contract as the branches

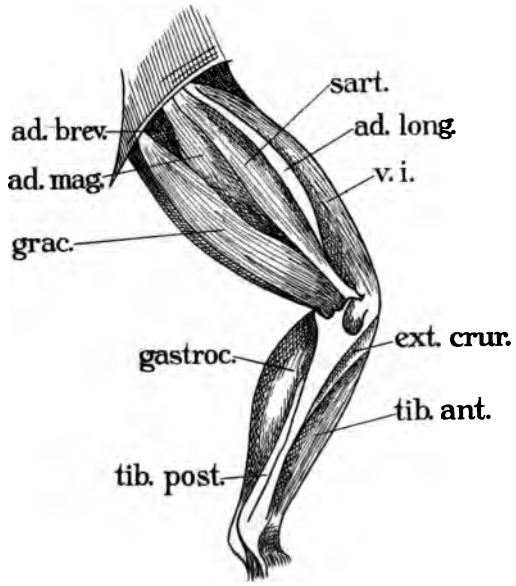



FIG. 2.—Anatomy of frogs leg; anterior view. (*After Ecker.*). ad. brev. = adductor brevis; ad. long. = adductor longus; ad. mag. = adductor magnus; grac. = gracilis; v.i., vastus internus; gastroc. = gastrocnemius; sart. = sartorius; ext. crur. = extensor cruris; tib. post. = tibialis posticus; tib. ant. = tibialis anticus.

going to them are cut. This is an instance of mechanical stimulation. Strip up the tube of skin that covers the gastrocnemius as if removing the finger of a glove. Tear through the loose connective tissue between the muscle and the bones of the leg, and divide the latter with scissors just below the knee. Cut across the thigh at its middle.

Place this nerve-muscle preparation on the glass plate.

## Stimulating Experiments on the Sciatic Nerve.

1. *Mechanical Stimulation.*—Cut off a small piece from the uper end of the nerve; during the cutting the gastrocnemius muscle contracts.

2. *Chemical Stimulation.*—Put a drop of concentrated saline solution on the free end of the nerve; in a short while the muscle begins to contract. Remove the piece which is moistened with the saline solution by cutting it off.

3. *Thermal Stimulation.*—The contact of a knitting needle with the nerve after the needle has been brought not quite to a red heat in the flame of a Bunsen burner has a stimulating effect on it.

It is necessary to bear in mind that these procedures destroy that part of the nerve to which they are applied, hence any subsequent stimulations must always be applied to new or uninjured parts of the nerve or muscle.

## Electricity.

**Fundamental Electric Conceptions necessary to an Understanding of Muscle and Nerve Physiology.**—A previous knowledge of physics is necessary for the understanding of the generation, conduction and application of electric currents, and the student is earnestly advised to make up any individual deficiency by a study of the chapter devoted to electrical batteries and currents in any of the numerous standard text-books on physics. Here we will limit ourselves to the definition of the more frequent technical terms used—for example: *Electrical potential, electro-motive force, volt and voltage,* the "*Ohm*" and *Ohm's law,* the *ampère,* etc.

Under ordinary conditions physical bodies are conceived to be in a state of electrical equilibrium; whether such an absoute electrical rest is physically possible or not is not our duty to discuss, but theoretically it is assumed. This *iso-electrical* state in anything is changed by chemical action, mechanic influences, heat, etc., etc., whenever these energies act on that thing. There occurs what is termed a change of "*electrical potential,* that

means, that instead of electrical equilibrium, *i.e.*, an iso-electric state in all parts of the thing, substance, cell, tissue, or organ we now have more electricity generated in one part than in another and an electric current passes from the part of the thing where the most intense chemic action takes place to the other parts. Some authors prefer to compare this difference of electrical potential to the difference of the level of water between a reservoir and its distributing pipes, a rather coarse analogy because it can give no conception of the origin of this difference. In a reservoir the energy is the force of gravity; in a cell-tissue or organ it is chemical action that causes this difference. To appreciate how electric currents may be generated in living cells and conducted through them the student should review his notes on the lectures concerning the theories of Van't Hoff, H. J. Hamburger ("Osmotischer Druck u. Ionenlehre," 1902), De Vries, and particularly of Arrhenius. An admirable English work to post oneself on these modern views of electrophysics is by Clerk Maxwell ("Introduction to Physical Chemistry") and also the work by Harry C. Jones on "The Elements of Physical Chemistry."

The terms used in speaking of the electrical current, its measurements and source are described in this place, because their definition should precede any experiments that require an electrical current or deal with electricity in some way. The letters E. M. F. stand for *electro-motive force.*

The unit of electrical pressure is the *volt.* It is about the amount of electricity produced by one Daniell cell. It equals the E. M. F. which steadily applied to a conductor whose resistance is one international ohm will produce a current of one ampère.

Voltage is that which tends to move a current over a conductor. Ampèrage is that which is moved. The water that flows over the falls of Niagara we might compare to ampèrage, while the distance it falls we would call voltage. The water in a river represents ampèrage; the swiftness with which it flows (caused by a difference of level), the voltage. We may have a very large river flowing slowly or a small stream running swiftly, and just so we have electrical currents of high ampèrage and low voltage.

The ampère is the unit of current (*C*) and is the amount of electricity that can be pushed through a resistance of one ohm by one volt of pressure. The ampère being more current than can be used for therapeutic or laboratory work, is divided for convenience into 1000 parts called "milliampères."

Resistance means that which opposes the passage of electricity through a circuit. The resistance of wires or other conductors varies directly as the length and inversely as their cross-section, and also inversely as their conductivity; a short wire offers less resistance than a long wire, and a thin wire offers more resistance than a thick one of the same length, much the same as a large pipe will carry more water than a small one.

Of conductors the metal silver is the best, but copper so nearly equals it that for all practical purposes it is to be preferred. Platinum has five times the resistance of copper. Metals being the best conductors of electricity, but any solution of the salts of metals decreases the resistance, as NaCl and $H_2O$. Review theory of electrolytic dissociation.

The ohm is the unit of electrical resistance (*R*) and is appoximately equal to the resistance offered by a piece of copper wire 250 inches long and 1/20 inch wide.

## Electrical Measurements.

About the year 1827 George Ohm gave us the law that bears his name and forms the basis of all electrical measurements —the strength of the current passing through any part of the circuit varies directly as the difference of potential between its elements, and inversely as the resistance of the circuit itself. This may be expressed in the following equation where $E$ = *E. M. F.* of battery in volts; $C$, current in ampères, which is sometimes spoken of as the "*intensity*," and $R$, total resistance in ohms.

$$C = \frac{E}{R}$$

A simple example is the flow of water through a nozzle of a syringe. The amount of water that passes through in a given

time will be directly proportioned to the force moving it and the resistance of the nozzle. The voltage of an electrical circuit represents the force moving the water and the resistance of the tube corresponds to the resistance of the wire. If we divide the former by the latter we have the quantity of water which flows through the nozzle in a given time, comparable to ampèrage.

Now if the nozzle of the syringe is longer (pressure same) less $H_2O$ would flow, or if the hole in the nozzle is made smaller the same would happen, because in both cases resistance is increased. Applying this to the electrical circuit we learn that the longer or thinner the conductor the greater the resistance and the less the flow of current.

## Application of the Law of Ohm.

If two electrodes from a galvanic battery be placed upon the body and a certain amount of pressure (volts) be turned into the circuit, a definite rate of current flow (ampèrage), will be established.

If we remove the electrodes to a part of the body where the resistance is greater, the same amount of voltage will not maintain the same current flow, but in order to obtain the original ampèrage we must either increase the voltage or decrease the resistance.

*Example.*—What is the current's strength when the E. M. F. of a cell is $=1.5$ volt, when the external resistance $R=0$, and when the internal resistance $=5$ ohms? What is the current strength when $R=100$?

## The Sources of Electricity.

The generation of an electrical current is an illustration of the law of the transformation of energy. According to the method by which we develop a current of electricity we speak of static, induced, and galvanic currents.

Static electricity is usually generated by the transformation of mechanical energy into electrical energy. It is of very high voltage and low ampèrage. Two glass disks revolved in opposite direc-

tions develop a current of static electricity by their friction, when their surfaces are rubbed by cushions of leather or silk during their revolutions.

Induced currents are those which have set up in a conductor by the movement about it of a natural magnet or of another conductor through which electricity is passing.

When we use a coil through which a current is passing to obtain the electrical field we may term this the primary coil. The coil which develops the induced current is then termed the secondary coil. The current flowing through the primary circuit is the *inducing current* and that produced in the secondary circuit is the *induced.* When the current is made or closed the induced current is *opposite* in direction to the inducing current. When it is broken or opened the induced current is in the same direction as the inducing current.

If we make one electro magnet (the armature) revolve about the other (the field magnet), we term the instrument a dynamo. In this case the current is developed by the transformation of mechanical energy.

If the field of magnetism is altered by causing an intermittent flow of electricity through the primary coil, we speak of the instrument as an inductorium. More information about induced currents will be given later in a description of the inductorium.

In the laboratory galvanic electricity is used, and it is obtained through the transformation of chemical energy into electrical energy in the electrical cell. In the construction of any electrical cell the essential parts are two electrically conducting plates which are unequally attacked by some chemical. These plates are usually made of two different metals, such as copper and zinc. Since it is only necessary that one plate be acted upon more than the other, we may use the same metal for each plate if we make the surface of one cleaner than the surface of the other. It is not requisite that the plates be of metal. Carbon, may be substituted for the plate designed to be acted upon little or not at all. The greater the difference in chemical action on the two plates the greater will be the difference of electrical potential, hence as

carbon is not affected by any chemical it is a very useful plate. The chemical often used is $H_2SO_4$, but $(NH_4)_2SO_4$ and other salts may be used.

*The Daniell Cell.*—In the type of cell which Daniell originated polarization is avoided. The cell consequently furnishes a current of unvarying strength. The two metals employed in the construction of a Daniell cell are zinc and copper. The copper is placed in a solution of copper sulphate kept saturated by crystals of the salt. The zinc is suspended in a porous cup filled with dilute sulphuric acid and the cup is placed in the copper sulphate solution. The zinc is amalgamated.

When a Daniell cell is connected with a galvanometer the zinc is the electro-positive element and the copper the electro-negative element, but the projecting end of the zinc, *i.e.*, the wire starting from it, is not called the positive pole or anode even though the zinc plate is the positive one. The wire starting from the positive plate is termed the negative pole or *cathode;* and that starting from the negative plate, the copper, is called the positive pole or *anode* (see Fig. 4, p. 12).

What is believed to take place in any galvanic cell is that the salts or acid in the electrolytic fluid—*i.e.*, the active agent in the liquid state—are split up into two different parts, called ions, and that these ions, charged with positive and negative electricity, respectively, unload these charges on the two plates, while an equalizing effect is brought about through the connecting wire. In other words, there is a constant disturbance of the electrical equilibrium through chemical action, and a tendency to re-establish the equilibrium, which tendency produces the current.

The projecting end or wire from the copper plate is known as the *positive electrode* or *anode*, while the end of the zinc is the *negative electrode* or *cathode* of the cell. A somewhat different terminology is used by Halliburton (see Handbook of Physiology, 9th Edition, p. 126) who follows Waller in distinguishing between *electro-positive* and *galvanometrically-positive* and between *electro-negative* and *galvanometrically-negative* and advises that physiologists adopt the nomenclature of the physicist.

Whenever the plates are connected by the conducting wire electricity passes from the anode, or copper, through the wire to the cathode, or zinc, outside of the battery and from the zinc to the copper inside the battery and through the liquid back to its point of origin. This continuous flow is called the *electric current*, and the different parts through which the current passes are known as the *circuit*. Whenever the connection is broken at any point the circuit is said to be open—no current flows; otherwise it is closed and current flows. If the circuit be open, both plates become charged with positive and negative electricity, respectively; but as there is no conductor to carry off these charges, further accumulation stops, and the chemical action ceases until the circuit is again closed.

Where the electricity leaves the solution (at the cathode) the copper ions are converted into metallic copper and deposited on the cathode. The quantity of zinc dissolved and copper deposited is proportional to the quantity of the current. An ampère is the quantity of current that deposits per minute 19.75 milligrams copper and dissolves 20.32 milligrams zinc, or when passed through a solution of silver nitrate deposits silver on the cathode at rate of 0.001118 gram per second.

It is to be observed that each metal is placed in a solution of its own salt. The ions carried to the respective poles are of the same nature chemically as the poles themselves, and hence do not set up opposing electromotive forces when they are de-ionized.

The current produced by the Daniell cell is almost perfectly constant, so long as $H_2SO_4$ still remains uncombined, and so long as the $CuSO_4$ solution is kept saturated. It may be remarked that the function of the porous cup it to prevent a deposit of copper on the zinc.

Whenever two or more galvanic cells are connected so as to use in one circuit the electricity generated by all the cells, the arrangement is spoken of as a galvanic battery; at present this term is likewise applied to a single cell.

*The Dry Cell.*—The source of electrical current used in the laboratory is the Leclanche cell (Fig. 3 ). It consists of a zinc

cup, which serves the double purpose of being the negative pole and forming the cover for the cell; and a carbon plate packed around with a mixture of powdered carbon and manganese dioxide, which plate is the positive pole. Separating the positive and negative poles is a layer of plaster of Paris saturated with ammonium chloride solution. The cell is sealed to prevent evaporation.

An electrical current is started when the carbon and zinc plates are connected by an electrical conductor; for instance, a copper wire. Within the cell the current flows from the zinc to the carbon plate and externally from the carbon to the zinc plate.

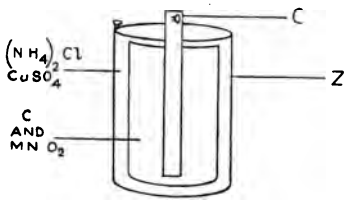



FIG. 3.—Dry cell.

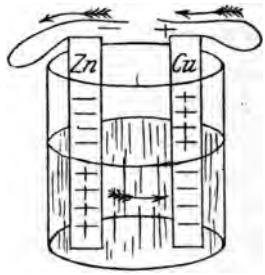



FIG. 4.

The current flows continually so long as the circuit is complete. When the cell is in action, the zinc forms a double chloride of zinc and ammonium while ammonia gas and hydrogen are liberated at the carbon pole. These cells should never be used continuously for many minutes, for they are rapidly polarized by the accumulation of H on the C plate. The unused cell regains its difference of potential by the union of the H with the O slowly given off by the $MnO_2$ which therefore acts as a depolarizer.

## To Increase Amount of Current.

*Coupling in Series.*—We can increase the electrical current by connecting together the poles of individual cells. If we connect the carbon pole of the one to the zinc pole of the other it is called coupling the cells in series. By doing this we increase the voltage.

Thus, if we connect four Daniell cells together in this manner, we get four volts and one ampère of current, one Daniell cell giving one volt and one ampère.

*Coupling in Multiple.*—When all the positive poles are connected by wires, and all the negative poles are connected by other wires, we have practically a single cell with plates as many times larger as we have taken cells. The electromotive force of a cell varies with its chemical constituents and not with the size of a cell. Now the internal resistance of a cell is inversely proportional to the size of the plates, so that by multiplying the size of the plates by the number of cells (take four as the number), the internal resistance is practically diminished one-fourth; increased quantity of current is therefore obtained. We get four ampères and one volt.

To obtain increased intensity of current with small external resistance as in a cautery wire, either large cells are used or a number of small cells are coupled together in multiple. With great external resistance, as in the application of the galvanic current to the human body, or the nerves of an animal, the cells are couplied in series, small elements being as good as large in this case.

**Description of Apparatus.** THE INDUCTORIUM. *The Primary Coil.*—This is wound with double silk-covered wire of 0.82 mm. diameter, having a resistance of 0.5 ohm, and is supported in a head piece bearing three posts and an automatic interrupter. The core consists of about ninety pieces of shellacked soft iron wire. This core actuates the automatic interrupter. The interrupter spring ends below in a collar with a set screw. By loosening the screw, the interrupter with its armature may be moved nearer to or farther from the magnetic core. Once set, the interrupter, will begin to vibrate as soon as the primary circuit is made. The outer binding posts are used for the tetanizing current. The left-hand outer post and the middle post are used when single induction currents are desired; they connect directly with the ends of the primary wire, thus excluding the interrupter. These several connections upon the

head-piece are simply arranged and are all in view; there are no concealed wires. These descriptions of apparatus, etc., are taken from the catalogue of the Harvard Apparatus Co.

From the head-piece extend two parallel rods 22 cm. in length, between which slides the secondary coil, containing 5000 turns of silk-covered wire 0.2 mm. in diameter. Over each layer of wire upon the secondary spool is placed a sheet of insulating paper. Each end of the secondary wire is fastened to a brass bar screwed to the ends of the hard rubber spool.

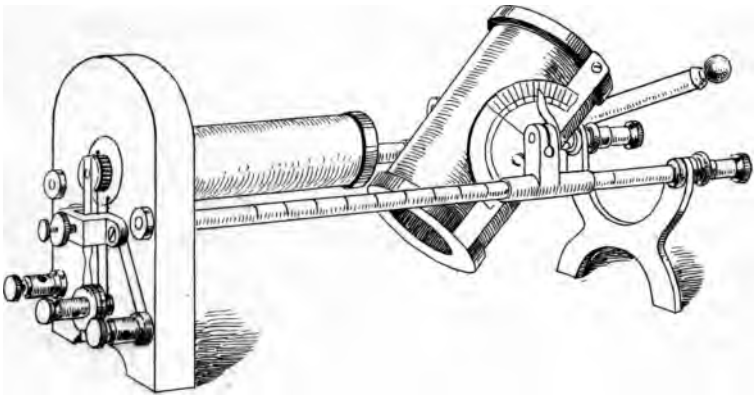


FIG. 5.—Inductorium. (*Enlarged from Porter's Introduction to Physiology.*)

The brass bar bears a trunnion which revolves in a split brass block, the friction of which is regulated by a screw. The trunnion block is cast in one piece with a tube 3 cm. in length, which slides upon the side rods. A set screw, not shown in Fig. 5, holds the trunnion block tube and the secondary spool at any desired point upon the side rods. This screw also serves to make the electrical contact between the trunnion block tube and the side rod more perfect. The secondary spool revolves between the side rods in a vertical plane. When the secondary coil has revolved through 90 degrees, a pin upon the side bar of the secondary coil strikes against the trunnion block and prevents further movement in that direction. One side rod is graduated in centimeters.

The side rods end in the secondary binding posts, so that moving the secondary coil does not drag the electrodes. Next the binding posts is placed a substantial "knife-switch" short-circuiting key with hard rubber handles.

**Platinum Electrodes.**—Stimulating electrodes with platinum points projecting about 10 mm., polished rubber handle 7.5 cm. long, and very flexible silk-covered connecting wires 65 cm. long, ending in nickel-plated brass tips. The rubber handle is in two pieces, screwed together, permitting easy access to the connection between the flexible wire and the stiff wire into which the platinum points are inserted.

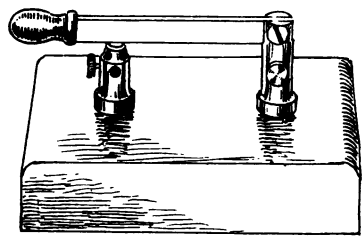


FIG. 6.—Simple key. (*From Porter's Introduction to Physiology.*)

**Simple Key.**—This consists of a copper bar with hard rubber handle pivoted at one end to a brass post with binding screws for electrical connection.

Near the other end of the bar is a platinum pin, which, when the key is closed, rests upon a platinum plate borne upon a second binding post. The base is of dark slate.

The contact bar is held partly by its own weight against the contact plate and partly by a wire spring not shown in Fig. 6. When it is desired to break the circuit the contact bar is turned back.

**Connections of the Primary Circuit.**—Three pieces of insulated wire bared at their ends, are used in connecting the elements in the primary circuit. Each one of the following is connected with a wire:

1. Post No. 1 of the inductorium with one of the poles of the cell.

2. The other pole of the cell with a binding post of the simple key.

3. The second binding post of the key with post number 3 of the inductorium.

When the circuit is closed with such connections a tetanizing current may be obtained from the secondary coil. Every time the primary current is closed and every time it is opened by the automatic hammer there is set up in the secondary circuit an induced current of very high voltage and very short duration. In closing the primary current an induced current is also set up in the primary coil opposite in direction to that of the primary current and so tending to weaken it, and on opening, it is in the same direction; the current in the secondary coil is therefore stronger on the break than on the make. The more horizontal the secondary, and the nearer the primary, the stronger the secondary current.

The *automatic hammer* consists of the electro-magnet, made up of the primary coil and the iron core described above and an interrupter which is interposed in the primary circuit; when this is broken, the core, no longer possessing magnetism, therefore cannot retain the interrupter against itself, for by virtue of its elasticity it springs back against the post from which it was drawn, and again closes the circuit. Thus the process is rapidly repeated until the circuit is broken by some external means. By the use of the hammer rapid makes and breaks of current are obtained.

## CHAPTER II.

### 1. Effect of Curare on Muscle and Nerve.

### 2. Rhythmical Contraction of Skeletal Muscle.

*Apparatus.*—Pithing needle, frog, scissors, knives, forceps, glass rod, frog board, glass plate, bowl, towels, normal saline solution, 1 per cent. solution of curare, small pipette, inductorium

wires, platinum, electrodes, dry cells, simple key, Biederman's solution, and beaker.

## 1. Poisoning of the Frog.

Description of Material: Curare Solution.—1 gram of the commercial dried curare dissolved in 100 cm. of water.

*Glass Pipette.*—A small glass pipette one end of which is drawn out to a fine point, and the other end of which is covered with a small rubber bulb.

EXPERIMENT.—Destroy the brain of the frog in the following way: holding the flank between the thumb and forefinger of the left hand, divide the medulla oblongata by inserting the point of a pin through the soft tissues covering the space between the skull and trunk, carefully avoiding all unnecessary injury. Introduce a wire seeker into the cranial cavity and destroy the brain. Arrest hemorrhage by packing the cavity.

Divide the skin longitudinally on the back of the left thigh. along the course of the sciatic nerve, for the space of an inch, Expose the nerve, taking care not to injure the femoral vessels. Lift the nerve gently with a glass seeker and pass beneath it a narrow tape moistened with normal saline solution. Bring the ends of the tape to the side of the thigh and ligate tightly at the upper third all the structures of the limb except the nerve. The left leg below the ligature is thus excluded from the blood supply, and will be known as the unpoisoned limb. A piece of filter-paper wet with normal saline solution should be laid over the nerve to keep it from drying.

The point of the pipette (which is now held with the right hand) is immersed in the curare solution. Draw up a few drops of the curare solution. Inject this into the dorsal lymph sac of the frog. This sac is found by making a small incision about 2 mm. long in the back of the frog, near the head. Wait until complete paralysis intervenes, which should be the case after one-fourth to one-half hour. The muscles of the left leg, however, will be found to be exempt from this general paralysis, for the muscle will respond to pinching of the toes.

Fix the hand electrodes to the binding posts of the secondary coil, with short-circuiting key closed. Expose both sciatic nerves from the vertebral column to the knees. Divide the skin covering each gastrocnemius muscle. Close the primary circuit, set the spring vibrating and open the short-circuiting key.

Stimulate the gastrocnemius muscle of both the poisoned and unpoisoned legs. Both contract. The paralysis is not due to injury of the right muscle, although supplied with blood containing curare.

Stimulate the right sciatic nerve. No contraction results.

Stimulate the left sciatic nerve as far above the ligature as possible; that is, in a region supplied with curarized blood. Contraction of the muscle at once follows.

Since the left sciatic, although supplied with curarized blood, is still functional, it follows that the curare has no influence on the nerve fiber. The inference is justifiable that the right nerve is also functional. The paralyzed portion must therefore be between the nerve trunk and the muscle fiber, that portion known as the "motor end-organ," unprotected by the neurilemma. At this point the blood and curare come into relation with the end-organ or synapse.

From this experiment it follows that muscles can be made to contract independently of nerves.

## Rhythmical Contraction of Skeletal Muscle.

Prepare Biederman's solution according to the following formula:

| | | |
|---|---|---|
| $NaCl$, | 5 | grams |
| $Na_2HPO_4$, | 2 | grams |
| $Na_2CO_3$, | .5 | grams |
| $H_2O$, | 1000 | grams |

Dip an end of the curarized sartorius muscle into Biederman's solution. The muscle is seen to contract rhythmically.

Read chapter on balanced and nutrient solutions in the Appendix of this volume.

## Recording of a Simple Muscle Curve.

*Apparatus.*—Pithing needle, frog, scissors, knives, forceps glass rod, frog board, glass plate, wires, towels, normal saline, bowl, dry cells, inductorium, key, signal magnet, adjustable stand, femur clamp, muscle lever, writing lever, piece of fine wire, muscle lever hook, scale pan, 10 gram weights, stand, tuning fork, cement, paper writing points, 3 clamps, kymograph and accessories, 1 per cent. solution of curare, and small pipette.

In this experiment it is intended to record the contraction of a muscle on a drum revolving at a moderate rate. The shortening is recorded in form of a curve. The advantages of this method are as follows:

1. It can be ascertained whether or not the shortening and stimulation are simultaneous.

2. It can be ascertained whether the period of contraction occupies a longer period of time than the period of relaxation or the reverse.

3. It can be ascertained whether the two periods occur with equal rapidity at different parts of their course.

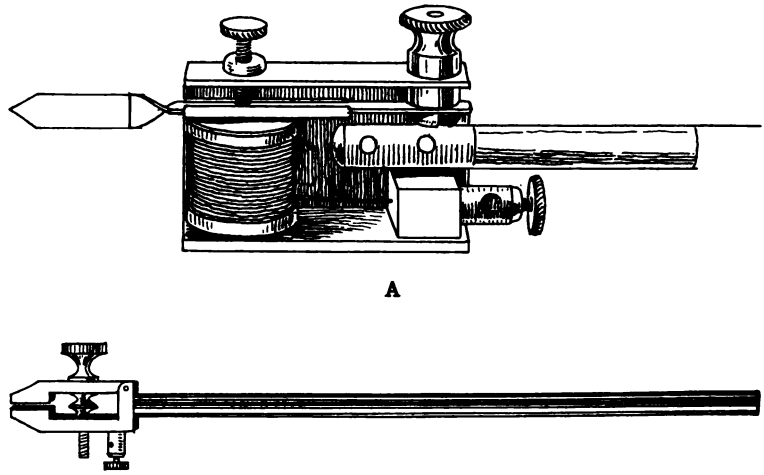
4. It can be ascertained whether the duration of the entire contraction is a measurable quantity or not.

**Description of Apparatus.** *Signal Magnet.*—A small metal box (Fig. 7, *A*) open at the front and ends, contains a strong magnet, the armature of which is mounted upon a steel spring. An accurate, fine adjustment screw regulates the excursion of the armature. One binding post is mounted upon the metal box, the other is insulated by a rubber block. This signal, in circuit with a vibrating tuning fork, will record 100 double vibrations per second. In the primary circuit of the inductorium it will record the make and break of the current without after-vibration. The handle is long enough to bring the writing point directly above or below the writing point of the muscle lever clamped to the same iron stand.

"Lag" due to residual magnetism is lessened or prevented by parchment paper shellacked to the under surface of the spring over

the core of the magnet. The paper should be renewed when necessary.

**Adjustable Stand.**—This is so arranged that by turning a screw the pole supporting the writing points may be turned in either direction, thus permitting of adjustment of the recording levers against the drum of the kymograph with ease.



A



B

FIG. 7.—*A*. Signal magnet; *B*. Flat jaw femur clamp. (*From Porter's Introduction to Physiology.*)

**Femur Clamp.**—This consists of smoothly working brass jaws attached to a steel rod. The separation and approximation of the jaws is effected by a spring and by a screw respectively. Objects of widely varying size can thus be held; for example, the femur of a nerve-muscle preparation or a board a centimeter thick. It has a binding post for making electrical connection with a muscle or other conductor held between its jaws (Fig. 7, *B*).

**The Light Muscle Lever.**—A stout yoke (Fig. 8) bears two set screws holding a steel axle upon which is mounted a light piece of tubing and a metal pulley. One end of the tubing tapers

slightly to receive the writing straw. The other projects behind the axle, and may be pressed upon by the accurately cut after-loading screw. The pulley is pierced with a hole for securing a fine wire, by means of which a weight may be suspended from the pulley when it is desirable that the weight should be applied near the axis of rotation. The muscle may also be weighed directly by means of a scale pan suspended from the double hook to which the lower end of the muscle is attached. If the tendon of the

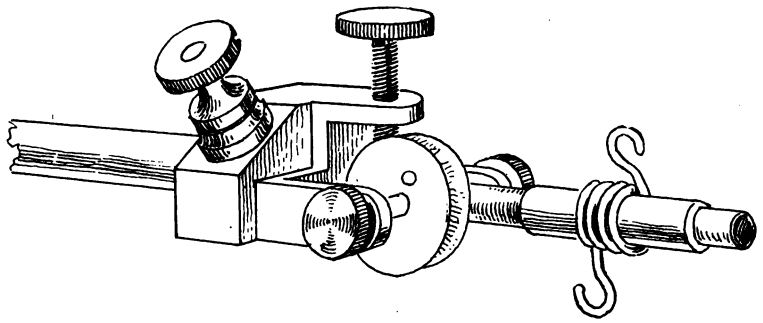


FIG. 8.—Light muscle lever. (*Enlarged from Porter's Introduction to Physiology.*)

muscle be fastened to the double hook by a fine wire, the free end of the wire may be carried to the insulated binding post provided for convenient electrical stimulation. The upper end of the muscle may be clasped in the femur clamp, and thus connected electrically with the binding post upon it. (Porter, in catalogue of Harvard Apparatus Co.)

**Writing Lever.**—A straight straw or a strip of aluminum, bent at one end to fasten with the double hook, pointed at the other, may be used in place of straw. If a straw is used it should be tipped with tin foil or parchment.

**The Extent of a Movement of the Muscle Lever.**—As the lever not only takes up and reproduces a movement, but at the same time magnifies it, it is essential that the degree of magnification be known, in order to determine the actual extent of the movement. The magnification of the lever is readily *determined by dividing the distance between the axis of the lever and its writing point by the dis-*

*tance between the axis and the point of attachment of the structure, and then dividing the height of the tracing by this quotient. The final quotient represents the extent of the movement.*

**Scale Pan.**—The muscles in the body normally do their work under a burden, which is that of the parts of the body which they move. To get the best work out of a muscle, it must therefore be weighted somewhat. For this purpose a slightly hollowed pan is usually burdened with a 10-gram weight and hooked to the muscle lever.

**Tuning Fork.**—A tuning fork giving 100 double vibrations per second is tipped with parchment or tin foil as a writing point. It serves to measure the latent period of muscular contraction and other physiologic phenomena of short duration.

**Tuning Forks Starter.**—A ⅂ shaped piece of brass provided with a handle fits closely upon the ends of the tuning fork. When the starter is smartly withdrawn, the tuning fork is thrown into vibration.

**The Recording Surface.**—The surface which receives and records the movements of a pen or lever is usually that of a cylinder which is covered with glazed paper and coated with a thin layer of soot, obtained by passing the cylinder through the flame of a gas burner. The axis of the cylinder is supported by a metal framework. If the writing point of the lever be placed against the cylinder and a movement be imparted to it, a portion of the soot is rubbed off, leaving a white line behind it. If the cylinder be stationary, the rise and fall of the lever are recorded as a vertical line. Such a record shows only the extent of a movement. If the cylinder is traveling at a uniform rate of speed, however, the rise and fall of the lever are recorded in the form of a curve, the width of the two arms of which will depend partly upon the rapidity of movement of the lever and partly on the rate of movement of the cylinder. The cylinder movement is initiated and maintained by clockwork. As the tracing is wave-like in form, the cylinder is frequently spoken of as a *kymograph* or wave recorder.

**Smoking of the Drum.**—In order to make a record it is neces-

sary to cover the drum with smoked glazed paper. Any pointed object, such as the aluminium tip, will, at the point of contact with the paper, make a white line by removing soot. The method of smoking the paper is as follows: Remove the drum from the kymograph by holding the upper framework of the drum with the right hand, and lifting the swing with the left. Should the drum not be held as directed, it may fall and cause injury to the instrument.

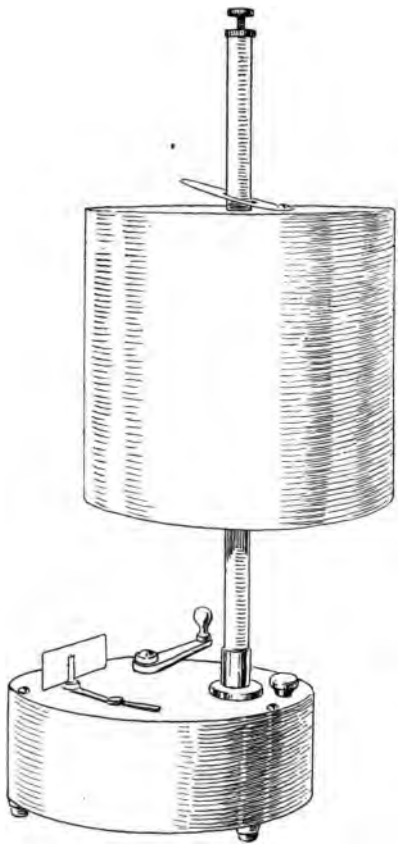


FIG. 9.—Kymograph. (*From Porter's Introduction to Physiology.*)

Place the drum across a strip of glazed paper and draw the ends tightly together. Moisten the mucilage at the end of the paper and fasten the ends firmly. Avoid folds or creases. Now hold the end of the drum in the right hand between the thumb and the first finger, and grasp the other end of the instrument in the same manner with the left hand. Turn the drum steadily at the speed of about one revolution a second, by keeping the fingers of the left hand still and turning with fingers of the right hand.

A gas flame is directed against the paper so that the flame is brought in contact with the surface of the paper at a point midway between the edge and the blue portion. Revolve the drum until it is covered uniformly with a thin coating. Trim overlapping edges of paper from the drum.

**Arrangements of Speeds of the Kymograph.**—The sleeve of the kymograph ends in a friction plate, which rests upon a metal disk driven by clockwork. Sleeve and friction plate revolve about a steel shaft which passes through both the heavy plates containing the clockwork, and is securely bolted to the bottom plate. The sleeve bears upon the steel shaft only by means of "bushings" at the ends of the sleeve, thus securing a bearing without "side-lash" and with little friction. As the sleeve with the drum rests upon the friction plate by gravity alone, it is easy to turn the drum by hand either forward or back, even while the clockwork is in action. This is a great convenience. At the top of the sleeve is a screw ending in a point which, when the screw is down, bears upon the end of the steel shaft and lifts the sleeve, and with it the drum, until the sleeve no longer rests upon the friction plate. The drum may then be "spun" by hand about the steel shaft. The impulse given by the hand will cause the drum to revolve for about one minute. The speed during any one revolution is practically uniform.

The clockwork consists of a stout spring about 6 meters in length driving a chain of gears. The speed is mainly determined by a fan slipped upon an extenion of the last pinion shaft in the chain. Four fans of different sizes are provided.

When the milled head shown in Fig. 9 to the right of the steel shaft is up, fast speeds are obtained. When the milled head is down, slow speeds are obtained.

These operations are easily and rapidly performed, though, as in all gear mechanism, an instant's pause is sometimes required to enable the gear teeth to engage. The clockwork should be in motion, without the fan, when the adjustments are being made.

With both fast and slow gearing, four fans of different areas may be used. They are slipped upon an extension of the last pinion shaft in the chain. Five slow and five fast speeds (exclusive of spinning) are thus obtained. An additional slow speed (50 cm. per hour) may be obtained with a very large fan. With one winding the drum will revolve from one to about seven hours, or

longer, depending on the fan employed. (From catalogue of Harvard Apparatus Co. and Porter, l. c.).

**Arrangement of Apparatus.**—1. *Arrangement of the Primary Circuit.*—(1) Connect the positive pole of the dry cell with post 1 of the inductorium.

(2) Connect the negative pole with one of the binding posts of the simple key.

(3) Connect post 2 of the inductorium with one of the binding posts of the signal magnet.

(4) Connect the remaining pole of the key with the signal magnet.

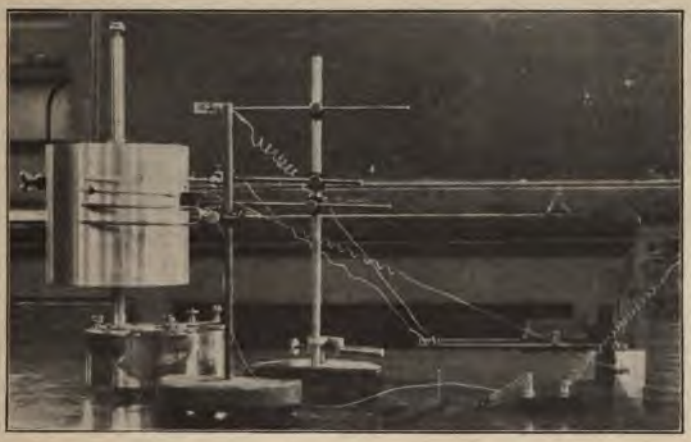


FIG. 10.—Arrangement of writing lever signal magnet and 1/100 sec. vibrating tuning fork to the kymograph for recording simple muscle curve.

2. *Arrangement of the Secondary Circuit.*—1. Prepare a nerve-muscle preparation. Fasten the femur in the muscle-clamp and connect the tendon with the tendon hook and the lever in the usual way with a piece of fine copper wire. Connect the muscle lever and the femur clamp with the secondary coil in the usual way. After-load the muscle with 20 grams. See that the lever is horrizontal. Push the secondary coil out some distance and find a position when only a minimal contraction is produced when the primary circuit is first made and then broken by the simple key.

3. *Preparation of the Recording Surface.*—Smoke a drum and first arrange it so it can be spun.

4. *The Grouping.*—(1) Holding the cylinder stationary, adjust the recording lever parallel to the cylinder and the writing point in contact with the paper.

(5) Now bring the writing point of the signal magnet a half inch directly below the point of the muscle lever.

(6) Have the tuning fork adjusted so that it will record the time of the contraction about half an inch below the record of the signal magnet.

**Recording.**—Set the drum revolving by spinning it moderately swift, with the mucle lever point and the tip of the signal magnet pressing against the paper. While the cylinder is revolving, make the primary circuit and press the end of the tuning fork against the surface. Remove the cylinder from the writing points, after erecting synchronous ordinates at the points of stimulation, the height of the contraction, and at the end of the relaxation.

The top line records the muscle curve or *myogram.* The curve may be divided into three portions:

1. A part that precedes the actual muscle contraction, *i.e.*, between the point of stimulation and the first evidence of the muscle shortening, known as the "latent period." This period is largely due to mechanical factors and varies in duration with a variety of conditions, *e.g.*, the kind of muscle, temperature, strength of stimulus, fatigue, inertia of lever, etc.

2. An ascending part, the contraction or period of increasing energy. This shows that the muscle at first contracts slowly, then rapidly, and then finally again slowly until the maximum point is reached.

3. A descending part, the relaxation, or period of decreasing energy. This shows that the muscle at first relaxes slowly, then rapidly, and then finally again slowly until the maximum point is reached.

The relaxation is succeeded by several residual or after vibrations, due to changes in the elasticity of the muscle.

The second line shows the exact time of stimulation.

The third line indicates the time that is consumed in the different periods of the muscle contraction, thus; 0.01 of a second for the latent period, which intervenes from the time the muscle is stimulated until it contracts, 5/200 of a second for the period of contraction, 15/200 of a second for the period of relaxation. From this it can be seen that the entire period occupies about 0.1 of a second.

The tuning fork may be removed and contractions recorded on the drum with slower speeds. First, the drum must be arranged for swift revolutions. Then the smallest fan must be placed on the extension of the pinion shaft. This gives a curve that is rather wide. By using the other fans in the same way, other curves narrower than this can be obtained.

### Effect of Fatigue on Muscle Curve.

Record a break contraction. Remove the writing point from the drum. Allow the drum to revolve. Stimulate the muscle nine times. Then replace the writing point against the drum. At every tenth contraction replace the lever against the drum very regularly until the muscle ceases to respond. What effect on the height of contraction is observed after each series of stimuli?

### Simple Muscle Curve Without Removal of the Muscle and Nerve from the Body of the Frog.

The term *myograph* refers to any form of apparatus that can be used in recording a muscle contraction graphically. The most frequently employed type of such an apparatus traces on the kymograph the muscle-curve or myogram.

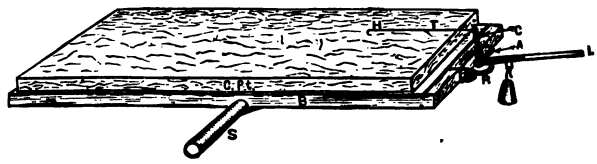
In all other previous myographs there were one of two defects; either the muscle was exposed to drying or removed from the circulation, or the moist chamber had been used; by which the drying can be avoided.

For the fundamental experiments on the elementary tissues, and for work with drugs in pharmacology, it is of very great importance to have a myograph that is simple and inexpensive and which

does not involve disturbing the circulatory system. The importance of the last point cannot be overestimated. The circulatory system not only brings a continuous supply of nutriment and oxygen to the muscle, but carries from the muscle the accumulating products of fatigue and thus enables the muscle to do much more work than would otherwise be possible.

In pharmacologic experiments it enables the experimenter to watch the influence of drugs carried to the muscle in the normal way, rather than applied in some artificial and abnormal way.

The frog-board myograph suggested by Winfield S. Hall is a new form of myograph so constructed as to permit all experiments usually performed on the gastrocnemius-sciatic preparation without exposing the active tissues to the atmosphere or disturbing the blood supply.




FROG BOARD MYOGRAPH.

FIG. 11.—*S*, The shaft which is clamped to the upright stand; *B*, the oaken base; *C. Pt*, the cork plate to which the frog is fixed; *A*, the lever axis and slide lever holder; *W*, the weight; *L*, the light lever about 20 cm. in length; *H*, the tendon-hook which is joined through the thread *t*, which passes through the eye and under spring catch (*c*); *R*, the lever-rest. (*From Winfield S. Hall, Journ. Am. Med. Assoc.*, Aug. 22, 1903.)

The instrument is constructed as follows: An open base about one-fourth of an inch in thickness supports a cork plate of equal thickness; the cork plate presents a surface about 10 by 25 cm. The lever holder at the end of the plate is constructed of thin sheet steel and slips form side to side in order to bring it opposite either leg of the frog.

The distance from the axis of the elbow-lever to the thread-eye is the same as that to the weight, therefore, the weight lifted by the muscle is the actual weight hung upon the weight link. When the lever passes a little below the horizontal position, it comes into contact with the rest. The rest can be used in "after-loading"

a muscle. For further description of the instrument, see the figure and its description.

In the use of the frog-board myograph one proceeds as follows: The frog is pithed and pinned, dorsum up, on the cork-plate, with the feet at the lever end. The tendo Achilles is exposed and loosened from the tarsal ligaments: the tendon-hook (*H*) is passed through the tendon and the length of the thread adjusted at *c*. The skin on the thigh is opened to the extent of 2 cm. and the biceps femoris muscle removed, the sciatic nerve carefully separated from the sciatic artery and placed on the insulated electrodes. Stimulation may be made from time to time for a period of several hours before the preparation becomes exhausted.

Take tracings of the frog's muscle contraction as in the previous experiments of testing the effect of fatigue upon muscle. Compare the tracings of the muscles outside of and within the body.

**The Influence of Load on Height of Contraction.**—Attach a curarized gastrocnemius muscle to the muscle lever and bring the writing-point against the smoked drum. Connect the binding posts on the lever and scale pan only; with the drum at rest record the contraction on stimulating with a minimal make induced current. Turn the drum by hand 1 mm.; continue to add 10 gram weights. Note the results. Up to a certain point the height is increased with each increment. This is soon reached and with each addition of 10 gram weights the height of the contraction diminishes, until finally the muscle will not be able to contract at all.

**Summation of Inadequate Stimuli.**—Place the secondary coil of the inductorium in such a position that the break current shall be nearly, but not quite sufficient to cause a contraction. Let the muscle rest without stimulation for about a minute. Repeat the inadequate single stimulation at intervals of five seconds.

After a time, contraction will be secured. No curve need be written.

The excitation outlasts the stimulus, and re-enforces subse-

quent stimuli; finally the summed excitations call forth a contraction. Summation is of frequent occurrence probably in all living tissues.

## CHAPTER III.

**Law of Contraction.**—The dependency of the irritation of motor nerves on the strength and direction of the constant electric current is to be investigated.

*Apparatus.*—Pithing needle, frog, scissors, knives, forceps, glass rod, frog board, glass plate, bowl, towels, normal saline, solution,

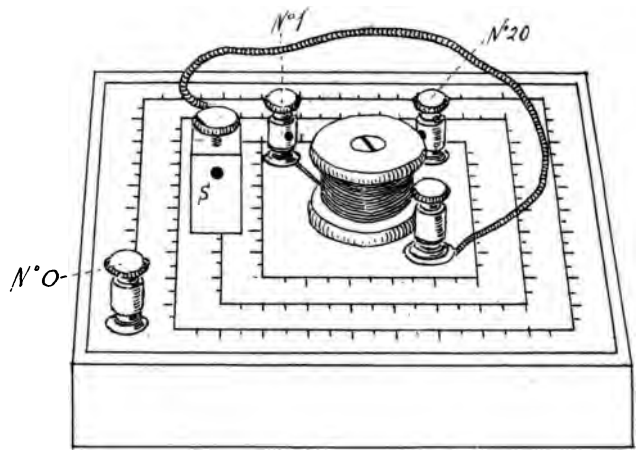



FIG. 12.—Rheocord, square model. (*From Porter's Introduction to Physiology.*)

5--20 dry cells, simple key, rheocord, rocking key non-polarizable electrodes, moist chamber, small femur clamp, stand, clamps, 2 zinc rods, concentrated zinc sulphate solution, dilute $H_2SO_4$, mercury, kymograph, light muscle lever, writing lever, piece of thread, scale pan, 10 gram weights.

**Description of Apparatus.**—Rheocord: Rheocords are instruments by means of which a current may be divided and a definite portion sent through a tissue. The rheocord, or potential divider, shown in Fig. 12, according to the description of W. T. Porter, is a block of hard maple 12.5 cm. square, upon which is placed

a centimeter scale beginning at the 0-post shown on the left side of the figure and ending at the 1-meter post visible in the back-ground to the left. Along the scale, between these two posts, is stretched the first meter of a continuous German silver wire, 0.26 mm. in diameter and 20 meters long. The remaining 19 meters of this wire are coiled upon a spool, and the free end is fastened to the 20-meter posts shown in the back-ground to the right of the spool. One of the posts may be turned, in order to keep the wire taut, in case changes of temperature have caused it to lengthen. The under surface of the contact block is bevelled so that the metal touches the wire only with one edge; the opposite edge is supported by a piece of hard rubber.

A flexible cable leads from the contact block to the binding post shown in the foreground to the right.

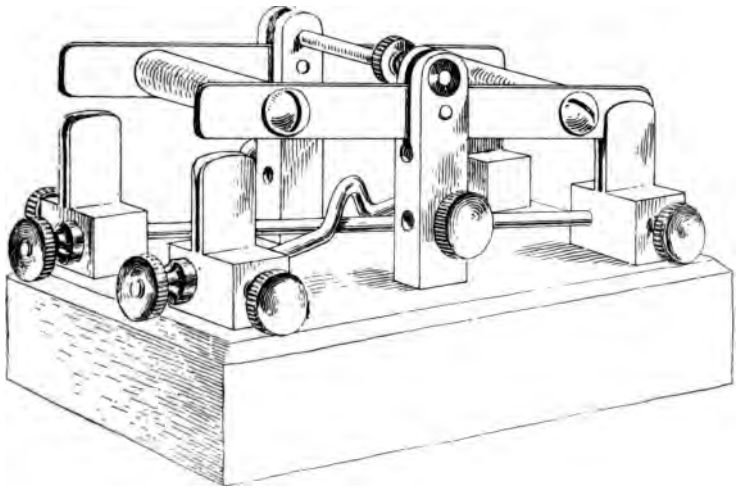


FIG. 13.—Rocking key. (*From Porter's Introduction to Physiology.*)

**Rocking Key.**—The instrument illustrated by Fig. 13 serves as a simple key, short-circuiting key, and pole changer. It is in fact a universal key. No mercury is used.

The central binding posts are prolonged upward and each is slotted to receive a brass bar which is pivoted in the slot by a

horizontal pin. The brass bars are held parallel by two rubber rods which serve as handles. When the bars are depressed to one side or the other, they engage between plates of spring brass set into brass blocks each of which carries a binding screw. Cross wires enter these blocks, as shown in the figure. At one end the cross wires are soldered into the blocks, thus making an electrical contact. The two blocks at the other end are perforated by rubber cores or "bushings" through which the cross wires pass. The cross wires, therefore, make no electrical contact with these blocks. When contact is desired, the screw borne on the head of each cross wire is turned until its face presses against the brass block outside the bushing. In this position the key serves as a pole changer, commutator or "wippe." (W. T. Porter, Science, Vol. XXI, 1905, p. 752.)

A brass bar unites the central posts. At one end this cross bar does not make electrical contact with the post, but passes through a rubber bushing clearly shown in the figure. Contact is secured by turning a screw upon the cross bar until the face of the screw presses against the post outside the bushing. When this contact is made, the instrument may be used as a short-circuiting key.

**Non-polarizable Electrodes.**—From his instruction on the theory of electrolytic dissociation the student should be familiar with the fact that when metal electrodes come in contact with an electrolyte in solution, polarization currents develop. Electrodes made of metal for this reason must be avoided in the study of the effect of the galvanic current on muscle and nerve. A "non-polarizable" electrode should be employed. Strictly speaking, no electrode is non-polarizable, but practically the polarization errors are excluded in the apparatus shown in Fig. 14.

These electrodes are boot-shaped, made of potter's clay, and were designed by Prof. W. T. Porter. The leg is pierced with a hole 28 mm. deep and 8 mm. in diameter in which is placed the zinc. The foot is 20 mm. long, measured from its juncture with the leg. In the foot is a well for normal saline solutions, the purpose of which is to keep the feet equally saturated.

The zinc has a nickel-plated screw for the fine wire which connects the zinc with the binding posts.

Fill the tubes about half full through a small pipette with concentrated zinc sulphate solution; immerse the rods of amalgamated zinc in the latter.

The zinc rods are amalgamated as follows: Place a few globules of metallic mercury in a porcelain dish. Add a drop of dilute sulphuric acid. Place the zinc rod in the dish and rub the mercury well over its surface.

The boots as used in the laboratory are as a rule mounted in rubber holders in the apparatus presently to be described as the "Moist Chamber." If they were not thus held in a non-conductor but in metal clips, the current would pass from the enlarged boot through the metal holder to the other boot and thus effect a short circuit and not pass through the nerve or muscle lying over the small wells in the foot of each boot.

**Moist Chamber.**—The moist chamber (Fig. 14) is an ingenious device to present drying of the nerve and muscle and consists of a porcelain plate which bears near the margin a shallow groove. In this groove rests a glass cover which for the sake of clearness is omitted from the figure. To the porcelain plate is screwed a rod, by which the plate may be supported on a stand. Within the glass cover are two right-angled rods. One of the rods carries a small clamp, composed of a split screw on which moves a nut, by means of which the femur of a nerve muscle preparation may be firmly grasped. The holder for the split screw is arranged to permit of motion in all directions. Both right-angled rods carry unpolarizable electrodes. Each of these is held by a hard rubber holder. These boots should be kept in normal salt solution. To the hole in the zinc plate is attached a wire which connects with one of the four binding posts shown in Fig. 12. These four posts are in electrical connection with four posts beneath the porcelain plate. The boot electrodes serve equally as well for leading off the nerves or muscle to the electrometer and for stimulation. After use the boots should be emptied, rinsed in tap water, drained and placed in several hundred cubic centimeters of

normal saline solution until wanted again. *If the foot of the boot is kept saturated with normal saline solution, these electrodes will remain non-polarizable.*

The *femur clamp used in this experiment* is the small one which is connected with the moist chamber.

Prepare a nerve-muscle preparation. Fasten the femur in the clamp of the moist chamber, connect the tendon with the lever

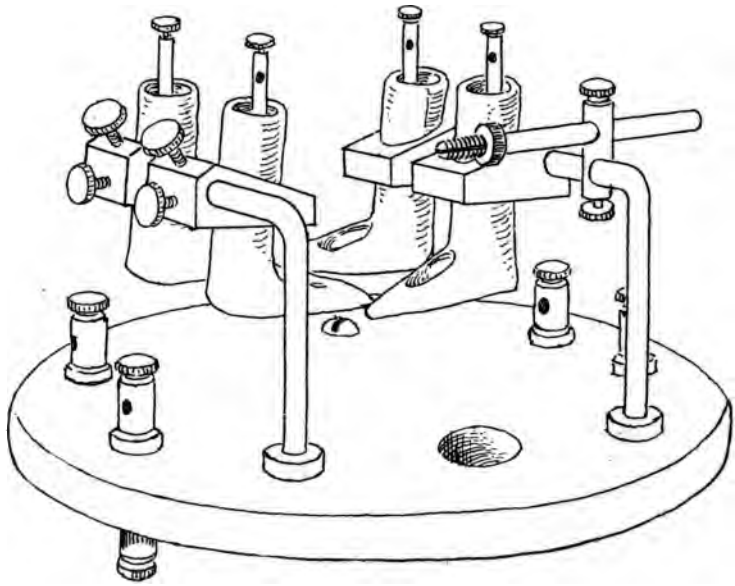


FIG. 14.—Moist Chamber. (*Modified from Porter's Introduction to Physiology.*)

after-load with 20 grams. Place the nerve carefully over the non-polarizable electrodes; moisten filter-paper with warm water and place it in the moist chamber near the nerve.

EXPERIMENT.—The effects produced in a nerve, as shown by the muscle contraction at the make and break of the current, will vary somewhat with the strength as well as with the direction of the current, *i.e.*, whether it is *ascending*, passing through the nerve *from* the muscle, or *descending*, passing through the nerve *toward* the muscle.

**Pflügers Law, Arrangement of Apparatus.**—Lead a wire from the negative pole of the cell to one of the binding posts of the simple key.

Lead a wire from the other pole of the key to post 1 of the rheocord.

Lead a wire from post of slider of the rheocord to an end post of the rocking key.

Lead a wire from positive pole of battery to the post of the rheocord.

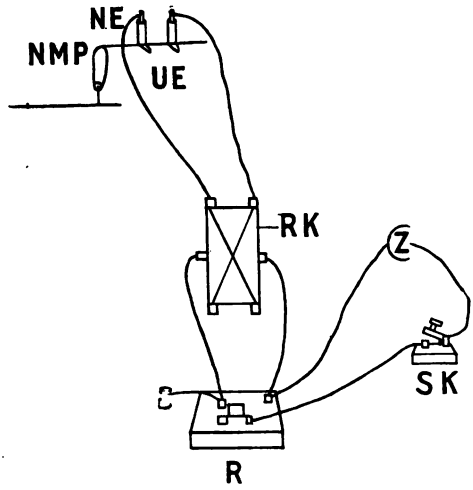



FIG. 15.—Arrangement of apparatus to demonstrate the law of contraction: *NMP*, nerve-muscle preparation; *NE*, needle electrodes; *UE*, unpolarizable electrodes; *RK*, rocking key; *R*, rheocord; *SK*, simple key; Ⓩ, carbon and zinc of battery.

Lead a wire from this post to the other end post of the rocking key.

Lead from the central posts of the rocking key two wires to the non-polarizable boots in the moist chamber.

Turn the rocker of the commutator so that the current will be ascending.

In this manner the rheocord, a means of obtaining a very weak current, a pole changer, which allows of the change from ascending to descending currents, and a simple key to make and break the current are obtained in circuit.

Place the slider *S* close to post o, so that practically no current will be passing through the nerve.

Close and open the simple key. If no contraction results, push the slider further along the wire until a contraction does occur at the make but none at the break.

Record the contraction.

A *descending* current is now obtained by throwing the rocker of the commutator in the opposite direction which reverses the current.

*Turn* the drum half a centimeter. *Make and break* the current; the same result is obtained.

A current of this strength is termed a weak current.

Tabulate the results according to the following schema, in which *M* stands for make and *B* for break.

| Strength of current | Ascending | | Descending | |
|---|---|---|---|---|
| | M. | B. | M. | B. |
| Weak | C | | C | |
| Medium | C | C | C | C |
| Strong | | C | C | |

*Increase the strength of the current* by placing the slider a little further along the wire. At a certain distance, varying with the irritability of the nerve, contraction occurs with both the make and the break of the ascending or descending currents.

A current of this strength is termed a medium current. To obtain the characteristics of a strong current it will be necessary to increase the strength of the battery current and preferably employ a fresh nerve.

The explanation of these results is found in the altered irritability and conductivity of a nerve caused by and *during* the passage of a constant current. At the cathode a state of increased excitability called *katelectrotonus* is developed and at the anode a state of diminished excitability and conductivity known as

*anelectrotonus.* The results of the preceding experiment can be concisely stated as follows:

The *appearance of katelectrotonus as well as the disappearance of anelectrotonus cause stimulation.*

## Electrotonus of Nerve.

PROBLEM.—To determine how the *irritability of the nerve at the two electrodes* is affected.

*Apparatus.*—Pithing needle, frog, scissors, knives, forceps, glass rod, frog board, glass plate, bowl, towels, normal saline solution, 5 dry cells, current changer, non-polarizable electrodes, moist chamber, small femur clamp, stand, clamp, zinc rods, concentrated zinc sulphate solution, dilute $H_2SO_4$, mercury, crucible, kymograph, light muscle lever, writing lever, piece of thread, inductorium, simple key, needle electrodes, filter-paper, scale pan.

**Experimental Arrangement.**—Two currents have to pass to the nerve:

1. The so-called *polarizing* current, changing the *irritability*
2. The so-called *stimulating* current, testing the *irritability.*

*Polarizing Current.*—Connect dry cell, current changer, non-polarizable electrodes, simple key.

*Stimulating Current.*—The same arrangement is used as in the second lesson (p. 25) with the following changes.

The *stimulating electrodes* are not used, but needle electrodes are substituted in their place. These are composed of two ordinary needles which are stuck through a cork which is of the size and shape to fit in one of the hard rubber clips of the moist chamber.

The *single induction shock* is used. Therefore connect posts 1 and 2 of the inductorium with the dry cell.

*Close* the short-circuiting key of the inductorium.

Arrange the rocker of the commutator so that the current will be *descending,* by placing the cathode nearest the muscle.

Place the needle electrodes in the holder nearest the muscle in the moist-chamber.

Place filter-paper moistened with warm water in the chamber.

Prepare a *nerve-muscle preparation* and fix it to the muscle clamp and lever. Place the nerve on the two pairs of electrodes, making sure that the nerve is in contact at both points of each.

Begin with the secondary coil far out, leaving the short-circuiting key open, and find that position which will give

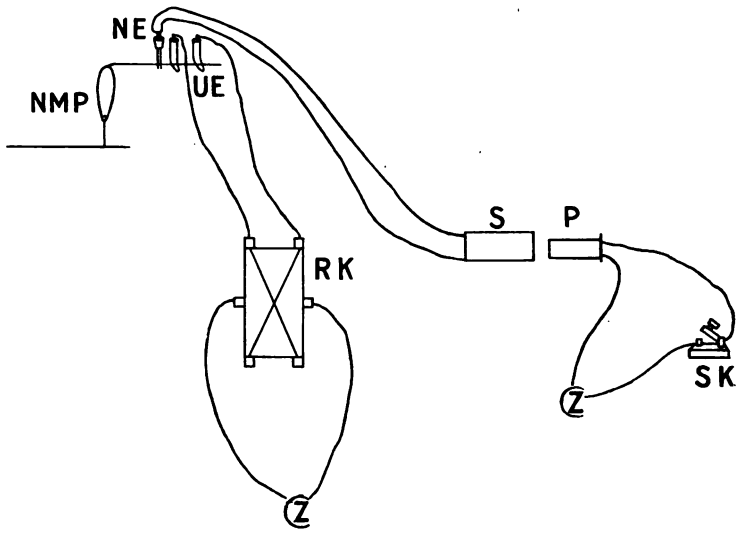



FIG. 16.—Arrangement of apparatus to demonstrate the experiment concerning the irritability of the nerve at the two electrodes: *NMP*, nerve muscle preparation; *NE*, needle electrodes; *UE*, unpolarizable electrodes; *RK*, rocking key; *S*, secondary coil; *P*, primary coil; *SK*, simple key; *Z*, battery.

an induced shock, just sufficient to produce the feeblest contraction when the primary circuit is made and broken with the simple key. With the kymograph stationary, adjust the writing points of the recording lever to the surface and record a contraction. Remove the writing-point. Return the key to its primary position.

Then close the polarizing circuit. The muscle contracts, but this it to be disregarded. Adjust the writing-point to the drum.

Stimulate the nerve again with the induced current of the same strength. The muscle contraction is now greater than before. Record a contraction after the passage of the current. The contraction is usually lessened.

During the passage of the constant galvanic current, therefore, the excitability of the nerve is *increased* in the neighborhood of the cathode, and correspondingly decreased in the neighborhood of the anode.

After the passage of the current the irritability is usually decreased in the neighborhood of the cathode.

Reverse the commutator or rocking key so that the polarizing current will be ascending, the anode nearest the muscle.

Repeat the steps of the preceding experiment, but increase first the strength of the induced current so as to be able to obtain a greater contraction. Thereafter on stimulating the nerve after the polarizing current is thrown into it, the muscle contraction will be less than before, or perhaps entirely absent.

After the passage of the current there results usually a small contraction when the nerve is stimulated in the anodal region.

During the passage of the constant galvanic current the excitability of the nerve is decreased in the neighborhood of the anode, and as previously shown, increased in the neighborhood of the cathode.

After the passage of the current there is usually a slight rise of irritability at the anode.

**The Metronome.**—The metronome, *M*, Fig. 17, like the tuning fork, is used to measure the time of physiological events. The instrument consists of an inverted pendulum, *P*, which is moved by clockwork at varying rates of speed depending upon the position of a sliding weight attached to it.

A signal magnet, *S*, which by electrical connection to a pendulum lever key, *R*, is attached to the base of the pendulum, records a mark upon a kymorgaph at each swing of the pendulum.

*The Electrical Circuit.*—One post of a dry cell, *C*, is connected through a key, *K*, to the rocking lever, *R*, which is supplied at both ends with a platinum contact.

A brass cup filled with mercury is situated beneath each contact and one of the contacts dips into its corresponding cup at each movement of the pendulum. The brass cups are connected, so when the lever makes contact at either one, the result is the same.

A single wire leads from the cups to one of the binding posts of the signal magnet. Another wire extends from the dry cell to the other post of the magnet.

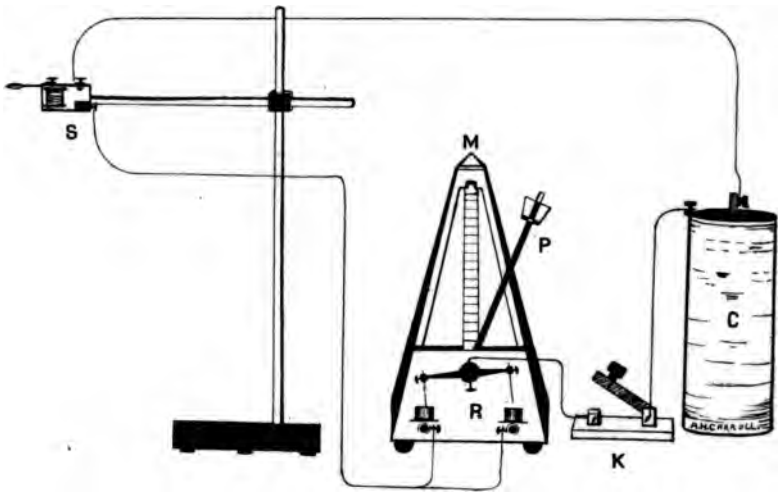



FIG. 17.—Arrangement of apparatus to record divisions of time measured by the metronome: *S*, signal magnet; *M*, metronome; *P*, pendulum; *R*, pendulum lever key; *K*, simple key; *C*, dry cell. (*A. C. Carroll.*)

PROBLEM.—To Determine: 1. How the conductivity of the nerve at the two electrodes is affected; 2. the seat of fatigue.

*Apparatus.*—Pithing needle, frog, scissors, knives, forceps, glass rod, frog board, glass plate, bowl, towels, normal saline solution, dry cells, 2 current changers, non-polarizable electrodes, moist chamber, small femur clamp, stands, clamps, zinc rods, concentrated zinc sulphate, dilute $H_2SO_4$, mercury, kymograph, light muscle lever, writing lever, piece of thread, scale pan, 10-gram weight, inductorium, simple key, 2 pairs of needle electrodes.

filter-paper, signal magnet glass slide, flexible slide holder, metronome.

**Experimental Arrangement.**—The apparatus is arranged as in the preceding experiment with the following exceptions:

Prepare a nerve muscle preparation with as long a nerve as possible.

A signal magnet is arranged in the primary circuit.

A pole changer should be placed in the primary circuit.

Two pairs of stimulating electrodes are arranged in the primary circuit between the two polarizing electrodes.

These stimulating electrodes are connected to the pole changer so that the current can pass through either one.

The demonstrator will sketch the schema on the blackboard.

Arrange the nerve on the polarizing electrodes and the stimulating electrodes nearer the muscle. Take a tracing. While it is being recorded, let a tuning fork record its vibrations beneath the point of the muscle lever. To mark on the abscissa of the muscle curve the exact moment at which the muscle was stimulated, turn back the drum until the writing point of the signal lies precisely in the line described by it when the current was broken. Now stimulate the muscle with another induction shock. The curved ordinate of the muscle lever will be synchronous with the ordinate of the signal.

Now remove the stimulating electrodes. In the same manner record a curve with the other pair of electrodes *away* from the muscle, in contact with the nerve.

After recording two clear tracings, first by stimulation of nerve near the muscle and second by stimulating most remote, determine the *latent period*, the time elapsing between the moment of stimulation, and the moment of contraction of the muscle.

The interval between the moment of stimulation, as recorded by the signal, and the beginning of contraction, is greater when the nerve is stimulated far from the muscle. The difference is the time required for the nerve impulse to traverse the length of nerve between the electrodes, provided of course that the interval between the arrival of the nerve impulse in the muscle and the

beginning of contraction is the same in both cases, an assumption considered reasonable by most physiologists.

Write now three other pairs of curves: one while a galvanic current passes through the non-polarizable electrodes in a descending direction (cathode nearest the muscle): a second while an ascending current passes (anode nearer the muscle); and a third, after the galvanic current has been broken for some minutes, as a control. During the writing of these curves measure the velocity of the drum with the tuning fork as before.

The speed of the nerve impulse will be found to be greater than normal when the nerve impulse starting at the second pair of metal electrodes passes through an extrapolar cathodal area and less than normal when that region is made anodal by reversing the galvanic current. In other words, the conductivity of the nerve has been increased by cathodal and diminished by anodal stimulation.

Conductivity is diminished by strong or protracted currents in the *cathodal* as well as in the *anodal* region.

Place two non-polarizable electrodes upon the nerve about 3 cm. apart. Connect them through a pole-changer with two dry cells. In the middle of the intrapolar region place two stimulating electrodes close together. Connect one of the stimulating electrodes directly to the secondary coil of an inductorium arranged for single induction currents. Lead from the other stimulating electrode to a piece of nerve or muscle about 4 cm. long, and thence to the secondary coil. The introduction of this great resistance will keep most of the polarizing current in the short bridge of nerve between the polarizing electrodes. Without this resistance, the polarizing current would pass through the stimulating circuit in preference to crossing the nerve between the stimulating electrodes. Observe that the nerve impulse created by the stimulus must pass through the cathodal region, if the current be descending, or the anodal region, if the current be ascending, in order to reach the muscle.

Find the position of the secondary coil at which the muscle will barely contract on *making* the stimulating current. Arrange

electrodes and the muscle, and produce a *make* closing of the polarizing current. Stimulate with a make induction current during the passage of the polarizing current. Open the polarizing current. After three minutes' rest, bring the cathode next the muscle and make the polarizing current as before. Then stimulate again with a *make* induction current of the same intensity as before.

Contraction will be absent, or at most very weak. The impulse will be blocked in the cathodal region. In truth, during the passage of *strong* or protracted currents, the conductivity is more diminished in the cathodal than in the anodal region.

**Deductions.**—After repeated clear records of these various methods of stimulation we are now in a position to account for the phenomena described by the law of contraction. The irritability of the nerve is increased at the cathode on closing, and at the anode on opening the galvanic current. The rise of irritability stimulates the nerve. The rise at the cathode is a more effective stimulus than the rise at the anode; consequently with weak currents the first stimulus to produce contraction is cathodal, *i.e.*, at the closure of the circuit. As the current intensity is increased, the anodal rise becomes also effective, and contraction is secured by both making and breaking the current.

But we have to deal also with a *decrease in irritability*, and still more important for the explanation of strong currents, with a *decrease in conductivity*. The irritability and conductivity are decreased on closure at the anode and on opening at the cathode. If the anode is nearest the muscle, the decrease in conductivity on closure of a strong current will block the nerve impulse coming from the cathode; it will therefore never reach the muscle, and there will be no contraction on closure. If the cathode is nearest the muscle, the conductivity may be so decreased on opening that the nerve impulse coming from the anode may be blocked. The decrease at cathode when the current is broken, is, however, less marked than the decrease at anode when the current is made, so that the cathodal decrease, even with strong currents, sometimes fails to block the impulse entirely. In that case, a weak

contraction may be obtained at the break of the descending current.

**The Seat of Fatigue.**—1. Expose a frog's sciatic plexuses as in making a nerve-muscle preparation. For convenience of stimulation place the glass rod beneath the left sciatic plexus. Stimulate with a tetanizing current through hand electrodes the upper end of the spinal cord until fatigue is shown by relaxation of the leg muscles.

Quickly place the electrodes on the raised sciatic plexus. What has been fatigued?

Disconnect the hand electrodes and connect the wires of the secondary circuit with the two central posts of the rocking key, from which the cross wires are removed. Run wires from two of the end posts to platinum electrodes in the moist chamber; and wires from the two remaining posts to the moist chamber for direct stimulation of the muscle.

2. Now prepare two similar nerve muscle preparations. Place one preparation in the moist chamber and weight with the muscle lever, scale pan and 10-gram weight, arrange the drum revolving at slowest speed against the point of the lever. Take a time tracing in thirds of a second beneath the tracing. Stimulate the preparation indirectly with the weak tetanizing current until the muscle relaxes. Pass the same current through the muscle until it is fatigued. Notice the time it takes to fatigue the muscle directly and indirectly.

3. Remove the nerve muscle preparation and disconnect all the wires. Connect a pair of non-polarizable electrodes, placed next to the femur clamp with a dry cell, interposing a rocking key and rheocord.

Arrange the needle electrodes, through which the tetanizing current should be led, as in the experiment on conductivity, between non-polarizable boots.

Place a fresh nerve-muscle preparation in the moist chamber. Lay the nerve over both pairs of electrodes. Weight the lever with 20 grams and attach the muscle tendon.

Block the conductivity of the nerve with the constant current.

Record the time in thirds of a second on the drum. Stimulate the nerve with the tetanizing current for a length of time equal to that necessary to fatigue the other preparation. Without discontinuing the stimulating current, remove the polarizing current. Does contraction now take place? What was fatigued in the indirect stimulation of the second part of this experiment? What is fatigued first, nerve cells, nerves, nerve endings or muscle? If not sure of the answers go over the entire experiments again.

**Polar Fatigue.**—Place a sartorius muscle on non-polarizable electrodes. A muscle can be fatigued only in a limited area; for instance, only around the cathode by repeatedly opening and closing the galvanic current. Closure will eventually be followed by no contraction; if the muscle is then tested by single induction shocks it will be found irritable except at the cathode. The fatigue is therefore polar or local.

## Du Bois Reymond Law of Excitation.

Du Bois Reymond has formulated certain laws governing the response of muscle tissue to the galvanic current.

1. The voltaic or galvanic current stimulates only when its intensity is suddenly and sufficiently increased or diminished, but not while it remains constant.
2. The stimulation occurs only at the cathode at make.
3. The stimulation occurs only at the anode at break.
4. The make is stronger than the break contraction.

*Apparatus.*—Pithing needle, frog, scissors, knives, forceps, glass rod, frog board, glass plate, wires, towels, bowl, normal saline, dry cells, non-polarizable boots, moist chamber, small femur clamp, stands, clamps, zinc rods, concentrated $ZnSO_4$, dilute $H_2SO_4$, mercury, dry cells, simple key, 1 per cent. solution of curare, small pipette, inductorium, large femur clamp, muscle lever, wires, kymograph, scale pan, 10-gram weights.

Place two non-polarizable boot electrodes in rubber holders upon a mounting-rod. Fill the boots half full of saturated solution

of zinc sulphate. Fill the well in the toe of each boot with normal saline solution. Place well amalgamated zincs in the boots and connect them through an open simple key with the poles of a battery. Prepare a sartorius muscle from a curarized frog, preserving the pelvic and tibial attachments. Lay the muscle upon the toes of the boot electrodes. Close the key.

The muscle will twitch when the current is made and probably when it is broken, but during the passage of the current there will be normally no contraction.

### Polar Stimulation of Muscle Making the Contraction Wave Visible to the Unaided Eye.

1. Slit the curarized sartorius muscle trouser-like from the lower end. Lay each end on a boot electrode. Make and break the current.

On making the current the cathodal side will contract; on breaking, the anodal side.

2. Lay the muscle on ice covered with a small piece of paraffin paper, to shield the muscle from water. When thoroughly cold, place the muscle in the Gaskell clamp, making very gentle pressure across the middle, and bring the non-polarizable electrodes against the ends. Make and, after a minute, break the current.

The excitation wave passes so slowly through the cooled muscle that the contraction can be seen with the unaided eye to begin at the cathode on closing and at the anode on opening the circuit.

## CHAPTER IV.

**Capillary Electrometer.**—Polarization current; Galvani's experiment; electromotive phenomena of muscle and nerve; polarization current (in nerve).

*Apparatus.*—Platinum foil, $CuSO_4$, pole changer, two dry cells, rheocord, capillary electrometer.

Pithing needle, frog, bowl, scissors, knives, forceps, glass rod,

frog board, glass plate, towels, normal saline, stands, clamps, moist chamber, four non-polarizable boots, simple key, capillary electrometer, Daniell cell, rheocord, inductorium, wheel interrupter, rod with copper hook at end, zinc rod.

## The Capillary Electrometer.

This is an instrument designed to detect and measure very feeble electrical variations that occur during the functioning of various tissues and organs particularly of muscles and glands.

In its construction two physical properties of mercury are made use of, first this metal is not only acted on by gravity but also by

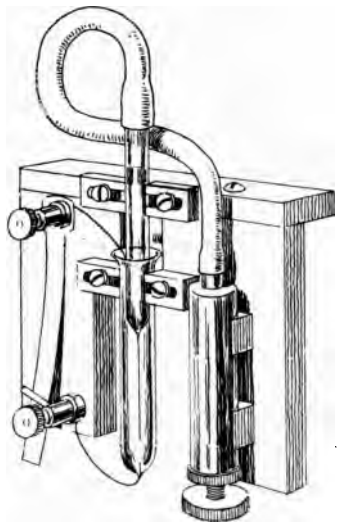


FIG. 18.—Capillary (stage) electrometer. (*Modified from Porter, Science*, 1905, xxii, p. 602.)

a second form of energy designated as *surface tension*; it is this force that keeps a drop of mercury spherical. Secondly, the surface tension can be altered by electricity. The idea in this instrument is to produce a column of mercury so fine that the most delicate variations in any current made to pass through

it can be detected by alteration in the surface tension of the column. What I speak of here as a column is in reality only a capillary tube filled with mercury, the tube being so fine that it requires a strong magnifying lens or a low power of microscope to detect the fluctuations of the mercury. When this metal is drawn into such a tube it does not flow through it but its surface tension is so great that the upper and lower meniscus is convex instead of concave as might be presumed. The glass capillary containing the Hg dips into a small vessel containing sulphuric acid; in the bottom of this vessel and underneath the $H_2SO_4$ is a second quantity of mercury. This metal in the capillary above and in the vessel below is connected with the source of the delicate current to be studied—whatever it may be—by two platinum wires. When such a delicate capillary of mercury is to be watched under the microscope it is necessary that we should be enabled to bring it into the field of observation by some accurate contrivance. This is in some types of electrometer accomplished by a pressure bottle or a syringe bulb. In the type perfected by W. T. Porter (l. c.) this is done by a metal cylinder in which a piston moves by a screw, thus pressure can be made on the mercury column. When the mercury in the capillary is pressed downward and then released again, a tiny portion of sulphuric acid is drawn up into the tube and in contact with the mercury. If as a consequence of gland secretion or muscle contraction, for example a current, should pass through the Hg and $H_2SO_4$, the surface tensions of the fluids are so changed that the meniscus of the mercury will move in the direction of the current. A graduation of the instrument may be effected by placing it in a circuit with currents of known strength and recording the degree of pressure needed to bring the meniscus back to its orginal position in terms of millimeters of mercury. A mercury manometer of finest caliber may be connected through a T-tube with the upper end of the capillary tube. This electrometer is so exceedingly sensitive that even the normal currents from the muscles of the fingers may deflect the meniscus and therefore it should be kept short-circuited; except during actual obser-

vation and in case of accuracy of measurement, the observer should work with rubber finger cots on all fingers.

Such slight variations as 1/10,000 of a volt can be measured by this instrument.

The most recent type of capillary electrometer offered by the Harvard Apparatus Co. is provided with a permanent short-circuiting key attached to it.

**Polarization Current.**—Connect a pair of non-polarizable electrodes in the moist chamber to the side cups of the pole-changer (without cross wires). One end pair of the pole-changer cups should now be connected with a dry cell. Turn the rocker to the opposite end posts to prevent the battery current from reaching the electrodes until it is wanted. The remaining pair of cups should be connected through a closed short-circuiting key with the capillary electrometer. Lay the sciatic nerve on the non-polarizable electrodes. Move the rocker to the other end posts to allow the galvanic current to flow some minutes through the nerve. Now turn the rocker back again and open the short circuiting key. The demonstrator will draw the schema on the blackboard.

Polarization of the nerve is indicated by a movement of the meniscus in a direction indicating that the former cathode is now positive to the former anode. The polarization current can be studied by the preceding arrangement, but in place of a nerve, two pieces of silver or platinum foil dipping in a solution of $CuSO_4$ will show the same phenomenon.

**Galvani's Experiment.**—Clamp to a stand a rod holding at its end a copper hook. Six inches beneath the rod attach a zinc rod to the stand by a clamp. Expose the sciatic plexus as in making a nerve muscle preparation, cutting the spinal column transversely one-fourth of an inch above the urostyle. Remove the skin from both legs.

Hang the preparation from the copper hook, introducing the latter between the sciatic plexus and the urostyle.

Swing the legs so as to effect contact with the zinc rod and observe the effect. Explain the result.[1]

[1] Review history of "Galvani's polemic with Volta. See Reference Luciani," *Physiologie d. Menschen*, Bd. III, S. 76.

## The Electromotive Phenomena of Muscle and Nerve.

**Demarcation Current of Muscle.**—1. Prepare a sartorius muscle; with a sharp pair of scissors cut off one end, connect one wire of the capillary electrometer with the center of the cut end and the other with the intact longitudinal surface of the muscle using a short-circuit key; a current will pass through the electrometer from the sound longitudinal to the cut surface (demarcation current or *current of injury*). At the cut surface the muscle substance decomposes and the chemical disintegration is associated with (perhaps) a negative current there—at least this part becomes negative to the intact part as can be seen by the deflection of the mercury meniscus in the electrometer.

It is known also that a contraction in a muscle renders the contracting parts negative to the part at rest. This is called the *action current.* Test it on the electrometer or an uninjured sartorius; then cut one end off and connect the muscle with the electrometer as above, note the degree of deflection on the micrometer scale, then open the key. If now the longitudinal surface is stimulated the intensity of the current of injury, as measured by the deflection of the meniscus, is decreased. The displacement is due to a difference of electrical potential that has been termed the *negative variation.*

The intensity of this current produced by stimulating an injured muscle will be less, the smaller the distance between the point that is stimulated on the longitudinal intact surface is from the cross-section.

The sartorius muscle shows these effects more strikingly when both ends are cut off and placed across non-polarizable electrodes connected with the capillary electrometer. The E. M. F. of the current of injury is about 0.07 volt; is it better designated as the current of *demarcation.*

**Compensation Method of Determining the E. M. F. of the Current of Demarcation.**—When a cut or injured sartorius muscle is so placed on non-polarizable electrodes that the negative electrode rests on the cross-section and a loop of thread is passed

over the muscle about 5 to 6 mm. from the cross-section and thence on to the positive electrode—the rheocord being arranged for weak currents—no closing contraction will occur when the key is closed. This so-called *polar refusal* is due to the following compensation of two currents; namely, (1) the current of demarcation in the injured muscle and (2) the galvanic current sent through it by closing the key. When the key is opened the galvanic circuit will be broken, but the demarcation circuit will still continue closed. It should also be noted that opening the key will produce a contraction; this occurs in accordance with the law of Du Bois Reymond stated before, *i.e.*, that any variation in the intensity of a current acts as a stimulus. On opening the

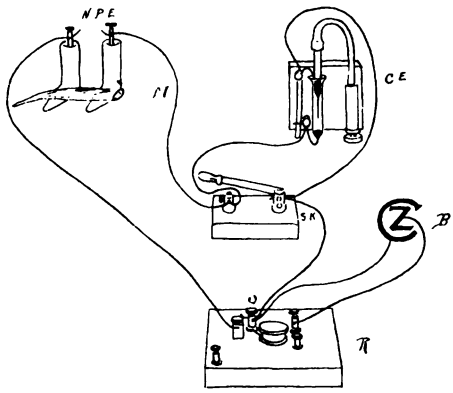



FIG. 19.—Scheme of compensation method: *R.*, rheocord; *CE.*, capillary electrometer; *SK*, simple key; *NPE*, non-polar electrodes; *M.*, muscle (cut end); *O* zero post.

key the artificial current and the muscle current are no longer compensated and hence the variation in electrical potential and the contraction. The E. M. F. of the demarcation current may be recorded in terms of fractions of any constant element; for example, a fresh Daniell cell. So much of the current of the cell is brought into the circuit with the current of injury, in an opposite direction however, as will just suffice to exactly offset the demarcation current. That means that in keying into circuit small portions of the E. M. F. of the Daniell cell it must

be done so cautiously that the meniscus of the electrometer is kept from moving in either a positive or negative direction. Detailed directions, see Fig. 19 which the demonstrator should enlarge and explain on the blackboard.

It is now presumed that the student is familiar with the use of the capillary electrometer and the rheocord. Cut off one end of a frog's sartorius muscle. The capillary electrometer is connected with a closed short-circuit key. The zero post of the rheocord is connected with the post joined to the mercury-filled capillary of electrometer, through the hinge side of the key. The remaining post of the key is connected with a non-polarizable electrode placed on the cross-section of the muscle, while the slider of the rheocord is joined to the other non-polar electrode on which the longitudinal uninjured part of the muscle is placed. Next the meniscus is adjusted into the field of vision and its position on the micrometer scale noted. The slider has been previous to this brought to the zero post. After the meniscus has come to rest the slider is moved along the rheocord until the meniscus has been made to return to its original position. Now note the number of millimeters between the positive post and the slider. This number when divided by 10,000 denotes the fraction of E. M. F. of the Daniell cell requisite to balance the demarcation current in the muscle; it averages 0.07 volt (from 0.04 to 0.09 volt.)

**Action Current of Muscle.**—Make a careful nerve muscle preparation from the sciatic and gastronemicus of the frog. Place the muscle on two non-polarizable electrodes in the moist chamber in such a way that the belly of the muscle comes on one electrode and the tendon on the other. Arrange the capillary electrometer so that it is connected with both non-polar electrodes by a closed short-circuit key and the tendon of the muscle is connected with the fine capillary of the electrometer. The nerve is placed on the electrodes from the secondary of an inductorium arranged for single make and break induction stimuli. Before stimulating at all open the short-circuit key and observe the meniscus of the electrometer; there will probably occur a deflection

due to the demarcation current of the muscle, for some slight injury is unavoidable in the preparation of it. Close the short-circuit key again. Now arrange the vibrating interrupter (of the Harvard Apparatus Co.) into the primary circuit. Adjust the meniscus into focus, then throw open the short-circuit key and now stimulate the nerve with single, slow and repeated induction stimuli; with each stimulus the meniscus will indicate a negative variation. The vibrating interrupter is a simple instrument to produce, apply and record, stimuli varing in number from one in a second to more than 150 per second. It is operated by a dry cell and recorded by a signal magnet.

In this interrupter a vibrating steel spring tipped at one end by a platinum wire dips into a mercury cup and by varying lengths of the spring the various rates of contacts are brought about. In Fig. 20 it is shown with the spring bent around on itself by a heavy weight; this is the arrangement to gradually effect one contact per second. In Fig. 21 the spring is straight or nearly so and arranged for 100 or more contacts per second. To be sure of absolute accuracy the interrupter ought to be adjusted to a Kronecker Chronograph or a fine metronome and the distances marked on the spring. I have found it a most useful piece of apparatus when once adjusted in this way.

**Threshold Value. Maximal and Minimal Stimuli.**—Prepare a gastrocnemius muscle. Arrange it to write on a smoked kymograph in this experiment and revolve the drum by hand; load the muscle with 10 grams. The secondary coil is so connected that one binding post leads to the muscle lever and the other to the muscle clamp. By turning the drum with the hand draw an abscissa. Begin with the secondary coil at zero and send a feeble break induction stimulus through the muscle. No contraction will result. Now gradually move the secondary close to the primary coil and repeat the break currents at each approximation. When a certain point has been reached the muscle will just contract and this represents the minimal stimulation and the minimal contraction. The amount of E. M. F. to produce this is termed the *threshold* value. At each approxima-

tion of 5 mm. of the second to primary coil, stimulate with another break current and record the contraction. The amplitude of these recorded contractions increases as the strength of

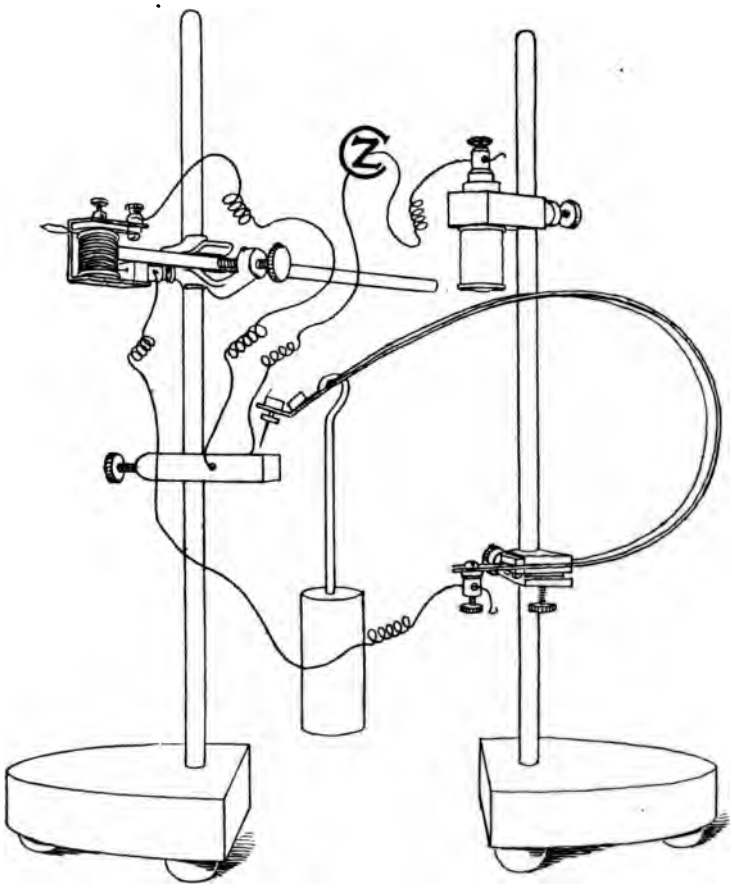


FIG. 20.—Vibrating interrupter arranged for one contact per second. (*Modified from Porter's Introduction to Physiology.*)

the stimulating current increases, at first rapidly, then less so up to a certain point, where further increase in strength of stimulus causes no increase in contraction. This is the point of maximal contraction and maximal stimulus.

**Demarcation Current of Nerve.**—Prepare a long piece of sciatic nerve with as little handling and injury as possible; place the cross-section on one electrode and the longitudinal intact part on the other non-polarized electrode, in the moist chamber. After connecting both electrodes with the capillary electrometer through a short-circuit key, bring the meniscus into the focus and open the short-circuit key. We observe here the same phenomenon as has been described in case of muscle. The capillary mercury meniscus indicates a current in the nerve from the intact long surface to the cross-section. The demarcation current of a nerve is not of as long a duration as in muscle nor near as strong. In muscle it has the E. M. F. of 0.06 to 0.07 volt, in the nerve only 0.025 volt. If a nerve trunk has attached to it several branches that have been injured or cut, the demarcation current of these branches may influence non-polarized electrodes placed on the main trunk and increase the deflection of the meniscus.

**Rheoscopic Muscle and Rheoscopic Frog.**—When a muscle or nerve becomes active electric currents are developed that can be measured; this much has been already studied. We now have to demonstrate that the E. M. F. of this action current can be led off from one contracting muscle to the nerve of a second nerve-muscle preparation and cause this to contract.

**Rheoscopic or Secondary Contraction.**—Prepare two gastrocnemius-sciatic nerve preparations and let us designate them as 1 and 2. With flat glass handles arrange the nerve of preparation 1 to rest on equator of No. 2. Stimulate the nerve of preparation No. 2 with a tetanizing current; both muscles will contract. In well prepared muscles simply snipping off a piece of nerve No. 2 with a hot pair of scissors will sometimes suffice. The second nerve-muscle preparation is stimulated by the action current of the first. It is stated by Herman that voluntary muscular contraction has not been known to produce secondary contraction.

A more complicated arrangement of apparatus is required if it is desired to demonstrate that the stimulus of the nerve of the

rheoscopic muscle is an action current from muscle No. 2. Then we need the moist chamber, non-polarized electrodes and the capillary electrometer with a closed short-circuit key. The stimulus is applied by means of the vibrating interrupter already described (Figs. 20 and 21). Muscle No. 1 is put on two non polarized electrodes (tendon on one boot, belly of muscle on

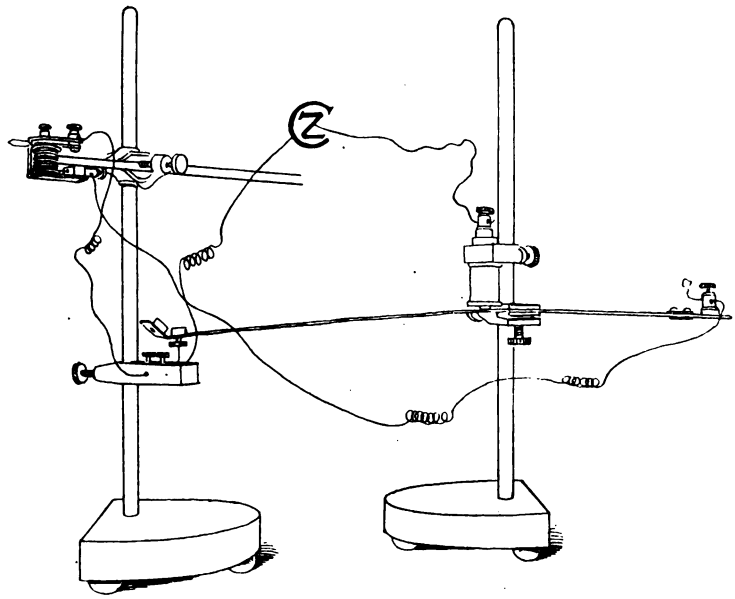


FIG. 21.—Vibrating interrupter arranged for 100 contacts per second. (*Modified from Porter's Introduction to Physiology.*)

other). The tendon is joined to the capillary of electrometer; the equator of muscle to the lower mercury vessel. Between the non-polarized electrodes and the electrometer insert a closed short-circuit key. Arrange the vibrating interrupter in the primary circuit and the meniscus into focus. Then open the short-circuiting key and note the oscillation due to the current of demarcation. Allow the meniscus to come to rest, then stimulate the nerve with single induction stimuli, note that as these are

repeated there will be a negative variation with each stimulus due to the current of action.

With the vibrating interrupter the number of stimuli per second may be sent into this preparation so rapidly that separate excursions of the meniscus are no longer recognizable and the eye perceives only a gray blur at the end of the capillary of mercury.

**The Stroboscopic Method** is designed for the purpose of making these rapid excursions of the meniscus visible. It consist only of a piece of thin black paper attached to the end of the signal lever, which is inserted into the primary circuit of the Du Bois Reymond inductorium. This apparatus must now be arranged for rapid tetanizing stimuli. The excursions of the paper occur each time the primary current is opened or closed by the vibrating magnet. Therefore they occur so rapidly as to produce the effect as if the paper were stationary. When this vibrating paper is brought next to the acid reservoir of the electrometer, so that the edge of the meniscus can be observed through the gray blur at the upper closed edge of the paper, the mercury meniscus will appear as clearly defined as if it were stationary. The stroboscopic paper and the mercury meniscus have the identical rate of vibration in that case. If the rates are different in these two, interference results, from which the rate of vibration of the observed back can be calculated. If the meniscus shows five vibrations per second, when observed through the stroboscope, its rate its five more per second than that of the stroboscope.

**Action Current of Nerve.** 1. *Negative Variation.*—Dissect out the nerve of a nerve muscle preparation and cut it close to the muscle. Lay the nerve in the moist chamber on non-polarizable electrodes placing the equator on one and a cross-section on the other. Connect through a short-circuiting key to the capillary electrometer. A second pair of non-polarizable electrodes is placed near the other cross-section of the nerve and connected with the secondary coil of an inductorium. The primary coil is connected through a key and the wheel interrupter with a dry cell. Now bring the meniscus into the field and open the short-

circuiting key. The demarcation current will displace the meniscus. Stimulate the nerve with induction shocks at different rates.

Every time the nerve is stimulated a negative variation will be observed.

2. The beginner may imagine that the current of action is dependent on the electrical stimulation, but as it can also be produced by mechanic stimulation it must be an expression of the changes in the nerve which constitute the nerve impulse.

From non-polarizable electrodes placed on the longitudinal surface and cross-section, lead to the capillary electrometer. Observe the position of the meniscus. Stimulate the nerve mechanically by pinching the end with forceps.

The negative variation will be observed as before.

## Velocity of Nerve Conduction in Frog and Man.

The experimental study of the conduction time of a nerve impulse in the frog was presented on page 41. At the temperature of 20° C. this rate is about 30 meters a second.

Charles D. Snyder has suggested an interpolation formula for calculating the velocity of nerve conduction in man. (*Science*, Sept. 29, 1911, p. 415.) Snyders' formula requires a knowledge of the use of logarithms. The formula he favors is the following:

$$\left(\frac{k_1}{k_0}\right)^{\frac{10}{t_1 t_0}} = Q_{10}$$

is substantially the same recommended by van Hoff, namely, $log_{10}k = a + bt$. Taking the body temperature of man as 37° C. and the value of $Q_{10}$ as 2.3 in both equations—since $Q_{10} = 10^{10-b}$ then $b = .0362$ and for the special case of frog $A = 0.753$, but for the special case of man then $log_{10}k = 0.753 + .0362 \times 37$ whence $k = 123.6$, from which the deduction is made that the velocity of nerve impulse in man is about 123.6 meters per second, which is in accordance with the conduction time in the median nerve of man calculated by Prof. H. Piper (Berlin) (*Arch. f. d. ges Physiol.*, 1908, Bd. CXXIV, p. 591) who gives 117 to 125 meters per second for human nerve.

# CHAPTER V.

## CIRCULATION.

### A Bloodless Method of Freeing the Frog's Heart (Njegotin's Method). *Zeitschr f. Physiol Technik,* June, 1910.

*Apparatus.*—Pithing needle, frog, bowl, scissors, knives, forceps, glass rod, frog board, glass plate, towels, normal saline solution, wires, silk ligatures, aneurysm needle.

The method of Njegotin is concerned with the freeing of the frog's heart without disturbing the circulation by loss of blood. With this end in view he separates the structure of the sternum, but leaves the suprascapula in place. Njegotin has tested this performance on numerous members of the Rana Esculenta class of frogs, after he had destroyed their brains and spinal cords. The following description of the technic concerns itself with the preparation of a large male curarized frog, belonging to the Rana Fusca class.

The operation is performed in the following stages:

1. Make a long incision through the skin, beginning 1/2 cm. distal to the cartilaginous part of the sternum, and ending nearly at the posterior border of the lower jaw-bone. Reverse the incision, coming backward to the shoulder-joint. One avoids bleeding in that one seeks under the raised, transparent skin a place for disconnection which is free of blood-vessels, after one has freed the musculus cutanei pectoris from the skin by the preparation needle.

2. The sternal (Fig. 22, IX, and Fig. 23, 4) and the epicoracoidal portions (Fig. 22, VI, and Fig. 23, 3) of the pectoral muscle, supplied with the superficial pectoral vessels, are doubly ligatured

and having been separated from above the coraco-radialis muscle are cut through at their surfaces of insertion into the sternum and epicoracoid cartilage, and displaced laterally.

The coraco-radialis muscle is separated from the episternal portion of the deltoid muscle, ligatured and plucked by forceps from the episternum and from the epicoracoid cartilage.

The coraco-clavicular artery and vein together with the coraco-radialis nerve are tied with a double ligature and divided. The coraco-brachialis longus muscle is ligatured near its attachment to the coracoid, divided and pulled to the side, but the brachial vein, at its antero-posterior border is not included in the ligature. The superficial part of the coraco-brachialis brevis muscle is treated in an analogous manner and separated from the coracoid; the vessels, which are branches of the coraco-clavicular arteries, are clamped or ligatured. Further dissection of the triangular deltoid muscle is made.

1. The episternal part is cut through at the episternum.

2. The clavicular part is separated from the lateral border of the clavicle.

3. The scapular part is incised at the anteroproximal part of the shoulder-joint.

A double ligation of the deltoid branch of the anterior dorsalis scapulæ artery, which is situated a little dorsal to the muscle, prevents bleeding.

4. In order to disarticulate the humerus, one makes longitudinal ventral and dorsodistal incisions in the capsule of the joint. One must separate with great care the dorsodistal region of the joint capsule and the intimately connected tendon of the scapular head of the anconeus muscle, in order to spare the adjacent subscapular vein and the subclavicular artery. Before incising the capsule of the joint prepare and divide the profunda portion of the coraco-brachialis brevis muscle.

5. One separates with a dull preparation needle the interscapular muscle, and incises with the small knife the synchondrosis which binds the scapula with the suprascapula. Press the vessel running at the anterior border of the synchondrosis prox-

imalward, but clamp the one on the ventral surface. The subscapular vein together with the subclavian artery and the brachialis longus inferior nerve are pushed distalward. The dorsalis scapulæ muscle is separated by means of the preparation needle from the lateral part of the suprascapula and from the medial segment of the scapula and laid distalward. Now

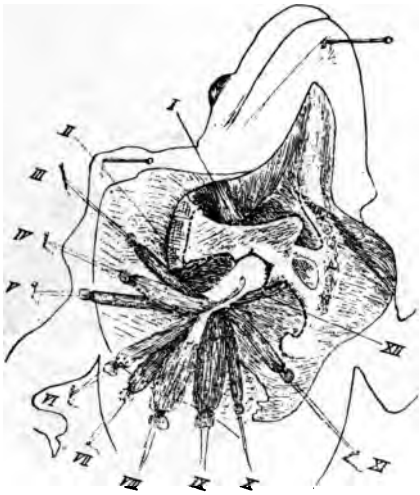



FIG. 22.—The shoulder girdle and a portion of the sternal structure have been freed after the preparation of the twelve muscles of the right side enumerated below, and they are represented by dotted lines. The dotted outlines besides this shows the direction of the incision which must be made for the disarticulation of the humerus and for the separation of the shoulder girdle (without the suprascapula) and the structure of the sternum. *I*, M. cucullaris; *II*, M. serratus inferior; *III*, Pars clavicularis; *IV*, Pars, scapularis; *V*, Pars episternalis; *VI*, M. pectoralis p. epicoracoidea; *VII*, M. coraco-brachialis longus; *VIII*, M. coraco-radialis; *IX*. M. Pectoralis p. sternalis; *X*, M. obliqus externus p. scapularis; *XI*, M. coraco-brachialis brevis p. superficialis; *XII*, M. coraco-brachialis brevis p. profunda.

loosen the cucullaris muscle and its vis-a-vis the serratus inferior muscle; the first close to the anterior border of the scapula, the second in the near neighborhood of the distal border of the same. Pay little attention to damage of the lateral branch of the cutanea magna artery which courses along the outer surface of the cucullar muscle, but protect from injury the subscapular vein and the subclavian artery. Now separate the sternohyoid at the

dorsal surface of the episternum, and pass an aneurysm needle threaded with silk around the coraco-clavicular artery and vein, and ligate the same. One next separates with the preparation needle or scissors the sternohyoid muscle from the dorsal surface of the cartilaginous and osseous portions of the sternum,

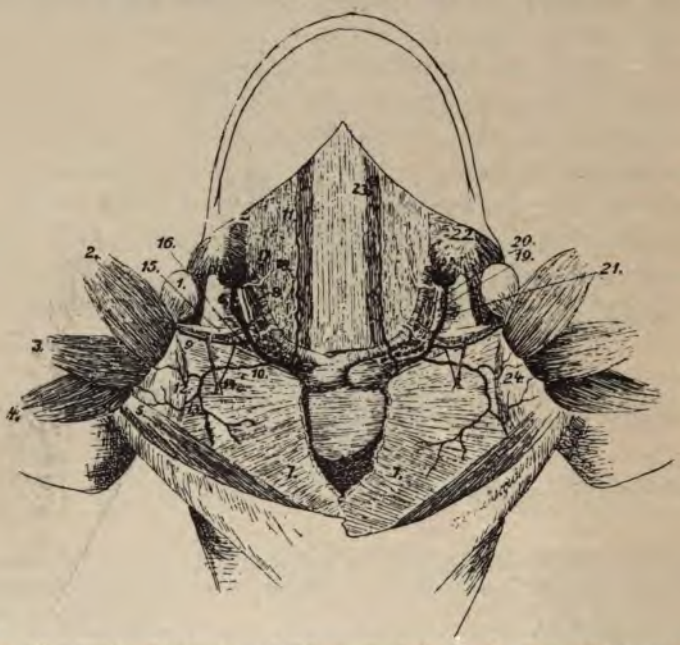



FIG. 23.—1. Caput humeri; 2. M. Coraco-radialis; 3. M. pectoralis p. epicoracoidea; 4. M. pectoralis p. sternalis; 5. M. pectoralis p. abdominalis; 6. Suprascapula with a part of the interscapularis muscle; 7. M. transversus; 8. M. petrohyoideus posticus tertius; 9. V. subscapluaris; 10. V. subclavicularis; 11. V. jugularis externa; 12. V. brachialis; 13. V. cutanea magna; 14. Ligature on the A. V. and N. coraco-claviculares; 15. N. brachialis; 16. A. subclavicularis; 17. N. glossopharyngeus; 18. N. hypoglossus; 19. N. vagus; 20. N. laryngeus longus; 21. V. jugularis interna; 22. Distal end of the lower jaw; 23. M. sterno-hyoideus (proximal part); 24. Skin.

and likewise from the middle end of the os coracoid bone. In this freeing of the sternohyoid muscle, the omohyoid muscle is in its caudo-lateral part also cut through. The middle portion of the rectus abdominis muscle should also at the same time be separated from the posterior surface of the cartilaginous sternal plate,

and the lateral portion likewise should be removed from its sternal origin.

6. The last cut, by which the heart is freed, must be so made that the abdominal vein is not injured; after a prick has been made with small, sharp scissors in the median incisure of the cartilaginous plate of the sternum, carry the incision right and left very close to the borders of the cartilaginous part of the sternum, to the bony portion of the sternum, and to the distal

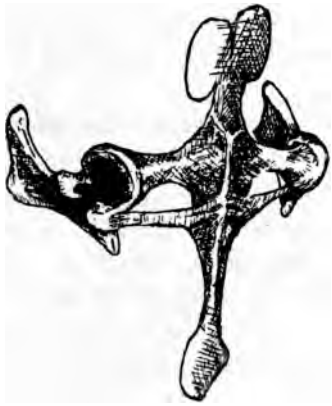


FIG. 24.—Sternal structure together with the shoulder girdle, the suprascapular having been allowed to remain in the body. The prox-lateral furrow of the cartilaginous part of the episternum has been purposely omitted from the figure.

border of the coracoid, in this manner separating the rectus sheath, the anterior lamella of the aponeurosis of the transversus muscle, the tendon of origin of the cutaneous muscles and the aponeurosis of the scapular portions of the obliquus externus muscles. Now all the ligatures are raised, and the shoulder girdle together with the structures of the sternum are separated; the heart with its vessels remaining in connection is exposed.

Fig. 23 is an enlargement of nearly twice the size of a preparation of a male Rana Fusca. The shoulder girdle with the exception of the suprascapula, and the sternal structures have been removed. The anterior extremities are turned out, whereby the skin on the lateral part of the transversus muscle has been

removed. The moderately stretched transversus muscle has been excised in its middle and distal portions. The incisions of the sterno-hyoid and the rectus abdominis muscles, which would run anterior to the heart, have been left out of the picture in order to make the sketch plain. The heart is in the pericardium. The internal jugular veins have been drawn to the side in order to show the vagus branch with the musculus petrohyoidei postici tertii. The dotted lines between the internal and external jugular veins and the two aortas sketch the further course of the cardiac branches of the vagus, but displaced ventro-lateral and distalward. The following parts are marked with arabic figures:

## Microscopic Observation of the Blood Circulation.

The circulation in the capillaries is observed very readily in any transparent part of a living animal by means of the microscope. The frog is the animal usually selected for the experiment, and the web and mesentery and sometimes the lung are the parts examined.

EXPERIMENT.—Curarize a frog and tie the animal back upward upon the frog board. Spread the web over the opening in the board. Do not spread the web too tightly or the circulation will be impeded. Examine the blood-vessels and blood elements under the lower power of the microscope. Note that some of the vessels pulsate and some do not. Observe the direction of the blood flow in the pulsating vessels is from large vessels into branches, while the flow in the non-pulsating vessels is from small branches into larger trunks.

With a higher power of the microscope examine one of the smaller vessels. As determined by the rate of movement of the blood elements, note the difference of the speed of the blood flow in the center and periphery of the vessel. Why do the white corpuscles seek the periphery of the stream?

Compare the red and white corpuscles in number.

**The Migration of the Leucocytes.**—Touch the web with the

point of a pin which has been dipped in strong acetic acid. Observe the effect of the irritant in the neighborhood of the spot irritated but select a spot that is fee from pigment and where capillaries can be distinctly discerned. Find one or more leucocytes as they adhere to the wall of the capillary. Presently, as the result of the irritant evolves one or more white curpuscles will be seen sending at first pseudopodia through the wall of the capillary and then extending more and more until the leucocytes are entirely outside of the little vessel and in the surrounding tissue. Observe that more and more white corpuscles move out in this way. This is called migration of white corpuscles and their accumulation in great numbers is called leucocytosis. Make a sketch of capillary and wandering white corpuscles in various positions and shapes. In the living organism this process is provoked by bacteria, injury by physical forces, heat, etc., etc. Bacteria are believed by Sir A. E. Wright to form a chemic substance which attracts the white corpuscles. This is termed *Chemotropism* or *Chemotaxis*. Wright found that washed bacteria do not attract leucocytes, but when they have been dipped or placed in the serum of that particular animal that is observed, they attract white corpuscles. Especially are white corpuscles attracted by bacteria that have been placed in serum of individuals that have already passed through that particular disease which the special bacterium that is observed causes. In other words, immune sera make the bacteria more tasty or attractive to leucocyte. The substance which is thus added to bacteria is called by Wright an *Opsonin*. Recently the idea is suggested that immune sera do not add but subtract something from the bacteria.

The accumulation of red and white blood cells which results as a consequence of local irritation or bacterial infection is really not a physiologic condition but it is pathologic, and called inflammation. When it is severe, the vessel walls change to such an extent that the red cells pass out as well as the white. The red ones, however, do not pass out because of any independent motion of their own, but because they are forced out by increased

blood pressure and because the so-called stomata of the capillary have been made wider by the stretching of the vessel. This is known as diapodesis.

These observations can also be made on the mesentery and the lungs of the frog. When the mesentery is used, exposure to air is sufficient to cause the irritation for inflammation.

## Direct Observation of the Action of the Frog's Heart.

*Apparatus.*—Pithing needle, frog, bowl, scissors, knives, forceps, glass rod, frog board, glass plate, towels, normal saline solution, wires, silk ligatures, aneurysm needle, watch glass or small bowl, heart lever, stands, clamps, wooden stool, recording apparatus, metronome, signal magnet, dry cells, simple key, normal saline at 37° and 5 °C.

Expose the frog's heart by Njegotin's method.

Note the relations of the pericardium to the heart and great vessels and to the surrounding viscera. Keep the preparation moist with 0.6 per cent. NaCl solution.

Although the frog is the most available animal, whenever it is possible, the terrapin, or at seaside laboratories, the heart of the dog fish, skate, or shark, is preferable. The heart of the large marine turtle (*Cavetta*) and also of some of the land turtles also make a very desirable object for such study.

*Simpler Method of Exposing the Heart of the Frog.*—If the frog is used, Njegotin's method should be carried out by the demonstrator to isolate the heart, but if the student has only two hours at his disposition, a simpler method will be sufficient. Tie the frog down on his back upon a cork frog board, spreading out the forelegs and hindlegs and pinning them to the board; pick up the skin in the middle line of the thorax with a toothed forceps; as the skin is pulled up slightly, cut a small hole into it with a pair of scissors; enter one blade of the scissors into this hole and cut upward toward the clavicles; at the level of the forelimb, cross this incision transversely with another incision; lay back the flaps of skin and with

a small dull-pointed scissors cut through the hyposternum at the root of this broad, heart-shaped cartilage; then with the dull blade of the scissors, longitudinally introduced, cut through the coracoid and the clavicle together with the muscles attached to and over them. This flap, consisting of bones and muscles, is pulled up with the forceps and then severed entirely. Now one sees the upper part of the heart covered by pericardium as well as the bulbus arteriosus with the two aortæ. In order to expose the heart entirely, the xiphoid cartilage must be cut out and the pericardium slit open.

## The Circulation in the Frog.

The ventricle receives arterial blood from the left auricle by the pulmonary veins and venous blood from the right auricle which receives the venous blood from the sinus venosus formed by the junction of the two vena cavæ superior and one vena cava inferior.

When the ventricle contracts it forces blood to the bulbus aortæ which divides into two aortæ. The aortæ subdivide into three branches on each side to supply 1, the head; 2, the lungs and skin, and the third branch unites with its fellow of the opposite side to form the dorsal aorta for the rest of the body.

Before opening the pericardium, note the rate of the heart beat, counting the number of beats in a minute.

Now open the pericardium so as to obtain a better view of the different pulsating portions of the heart and make the following observations:

Make the following observations on the heart:

I. Familiarize yourself with the position of the (a) sinus venosus, (b) auricle, (c) ventricle. Note the order in which these parts contract.

II. The change of color in the different parts during the contraction or systole and compare it with the relaxation or diastole.

III. With a stop-watch compare the time of the systole with that of the diastole.

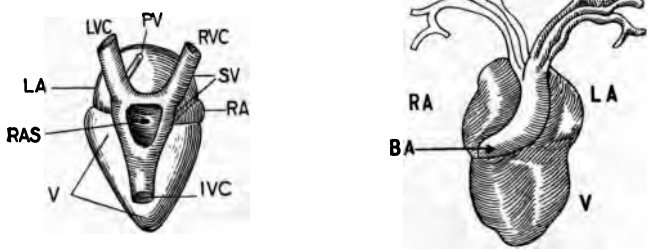
IV. Is there any change of form in the different contracting parts?

V. Is there any change of position?

VI. Is there any change in hardness?

VII. Count the rate of the heart beat per minute.

VIII. Tie two ligatures around the sinus venosus where it joins the large veins and also two ligatures around the bulbus arteriosus with the arteries that branch from it. With a sharp pair of scissors cut through each pair of ligatures. Remove the heart




FROG'S HEART.

FIG. 25.—A, Dorsal view: *RA*, right auricle; *LA*, left auricle; *V*, ventricle; *LVC*, left vena cava; *PV*, pulmonary vein; *RAS*, opening between right auricle and sinus venosus; *IVC*, inferior vena cava. B. Front view (*Ecker*): *RA*, right auricle; *LA*, left auricle; *BA*, bulbus arteriosus; *V*, ventricle. The bulbus arteriosus divides into right and left aorta.

from the thorax. Open the ligatures around the bulbus arteriosus and let the blood out. Open the ligature around the sinus venosus but with a threaded needle carefully pass a new ligature through the veins at this end and by this string suspend the heart in a small beaker of Ringer's solution. Now note whether all the observations made from I to VIII are still to be observed on the excised heart.

IX. After having counted the rate again, pack a beaker of Ringer's solution in ice. Count the rate. Take it out of the ice, place it in water at room temperature, about 70 degrees F. Count the rate again. Warm the water to 80 and 90 degrees and

observe the rate. As the heart can perform these functions outside of the body, what conclusions do you draw regarding the dependence of the work of the heart upon the extrinsic cardiac nerves and upon the central nervous system?

X. If the heart is still in good condition, sever the sinus venosus from the auricle. Suspend each separately in Ringer's solution. Does the sinus continue to beat? Is there any difference in the rate of the beat in the different parts of the heart?

XI. Cut the auricles from the ventricles by incising through the auriculo-ventricular groove. Note the results. Toward the end of the experiment, if the ventricle should have ceased beating, note whether it still responds to electrical stimuli.[1]

## The Graphic Method of Recording the Heart Beat.

**The Suspension Method.**—*Apparatus.*—Heart lever, stand, wooden stool, recording apparatus, dissecting appliances, frog board, etc.

**The Heart Lever.**—This is a very light lever. It consists of a nickelled brass rod supporting a brass axle 7 mm. in length. Attached to the axle is a very light aluminium wire 22 cm. long.

DETAILED DIRECTIONS FOR EXPERIMENT.—The method usually advised to bring the tip of the ventricle in connection with end of the heart lever involves the use of a bent pin or sharp copper wire or hook which is thrust through the apex of the heart. Most beginners ruin the heart in this effort, as they usually penetrate into the cavity of the ventricle. If the heart is still in connection with its vessels, the animal usually bleeds to death or the entire experiment is obscured by the issuing blood. In the turtle, there is a very convenient frenum attaching the tip of the apex to the diaphragm. It is best to pass a threaded curved needle around this frenum and tie the string tightly to this, rather than to the heart. In the frog, a very fine curved needle held by a needle holder, will save many a spoiled experiment. One of the best methods is to make a loop of fine thread and while one student holds this loop steadily over the apex, the

[1] Review "History of the Discovery of the Circulation of the Blood," by John C. Hemmeter, in *Johns Hopkins Hospital Bulletin*, Vol. XVI, May 1905, p. 170.

string itself lying on the upper surface of the ventricle, a second student grasps the tip of the ventricle with a fine forceps through the loop and as he slightly pulls the ventricle upward, the first student tightens the string on that part of the heart which is thus raised into the loop. In this way several loops can be put on the heart at the same time; in fact, in the turtle, and Elasmobranch fishes, one each can be placed on the sinus venosus, auricle and ventricle. When the heart has been secured at the ventricle in this manner, using a string about 10 inches long, the other end of the string is fastened to the heart lever by a globule of wax or a little cement. The tip of the lever, which is to write on the kymograph, is provided with a pointed piece of parchment paper or tinfoil. The movements must be recorded on a slowly moving drum. Beneath the record for the heart make a time tracing in seconds.

There is also a method of direct transmission for recording the heart contractions, which shall be described later.

If this experiment has been well conducted and without much loss of blood, the curve traced on the recording apparatus will be oblique near the apex, due to the greater work the ventricle has to perform in ejecting the blood into the aorta at the close of the systole; but if a hook has been used and much blood has been lost, the ascending limb of the heart curve will be vertical, almost like that of an ordinary skeletal muscle. A correct heart curve cannot be gotten from a wounded ventricle for this acts more like a gastrocnemius muscle.

## Dissection of the Extrinsic Cardiac Nerves of the Frog.

*Apparatus.*—Pithing needle, frog, bowl, scissors, knives, forceps, glass rod, frog board, glass plate, towels, normal saline, wires, silk ligatures, aneurysm needles, heart lever, stand, wooden stool, recording apparatus, dry cell, inductorium, simple key, platinum electrodes, time marker, shielded electrodes, needle electrodes, 2 per cent. solution of atropine sulphate, 10 per cent. solution of pilocarpine hydrochlorate, 10 per cent. solution

muscarin, 0.6 per cent. solution of cocaine hydrochlorate, saturated solution of digitalin, 1 per cent. solution of nicotine, 1 to 10,000 solution of adrenaline chloride.

*Directions.*—Expose the heart as in the previous experiments. Carefully cut away the sternum and muscles of the thorax. Locate the glosso-pharyngeal and hypoglossal nerves (see Fig. 26). Note the petrohyoid muscle along the anterior border of which is the vagus. In figure 26, *V* represents the branch of

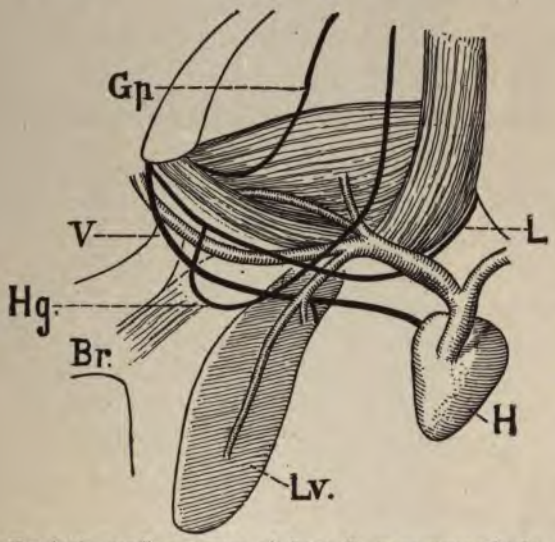



FIG. 26.—Extrinsic cardiac nerves of frog: *V*, vago-sympathetic; *Gp*, glosso-pharyngeal; *Hg*, hypoglossal; *Br*, brachial plexus; *L*, laryngeal nerve; *H*, heart; *Lv*, lungs. (*Busch.*)

the vagus leading to the heart. The trunk as thus exposed in the thorax is really a combined nerve, the cardiac branches of the sympathetic and the vagus uniting to form the nerve.

At first the vagus runs along the superior vena cava to the auricle giving off a branch to the lungs. Dissect out the vagus clearly, tie a very fine waxed thread loosely around it, and cut the nerve cephalad to the string. Divide the laryngeal branch also. Take care to avoid other nerves in placing the vagus upon platinum electrodes. Expose the heart by the simple method described.

Connect the ventricle by the method described with a light heart lever. The inductorium is arranged for weak tetanizing induction stimuli. The electric magnetic signal is linked into the primary circuit. See that the point of the writing lever is exactly above the point of the electric magnetic signal on the revolving cylinder which must be set to revolve at so slow a rate that the beats shall come close together but not so close that they could not be readily counted. Permit the ventricular contractions to be recorded for one minute. In marine laboratories the Elasmobranch fishes are convenient animals for experiments. For technics of experiments on these Selachii see foot note.[1]

## Stimulation of Vago-sympathetic Nerve.

1. Place the vagus nerve on the platinum electrode. Stimulate the vagus with a weak current for ten seconds. Is there any change in the rate or force of the heart beat? Should the stimulus be too feeble, increase the strength by reducing the distance of the secondary from the primary coil.

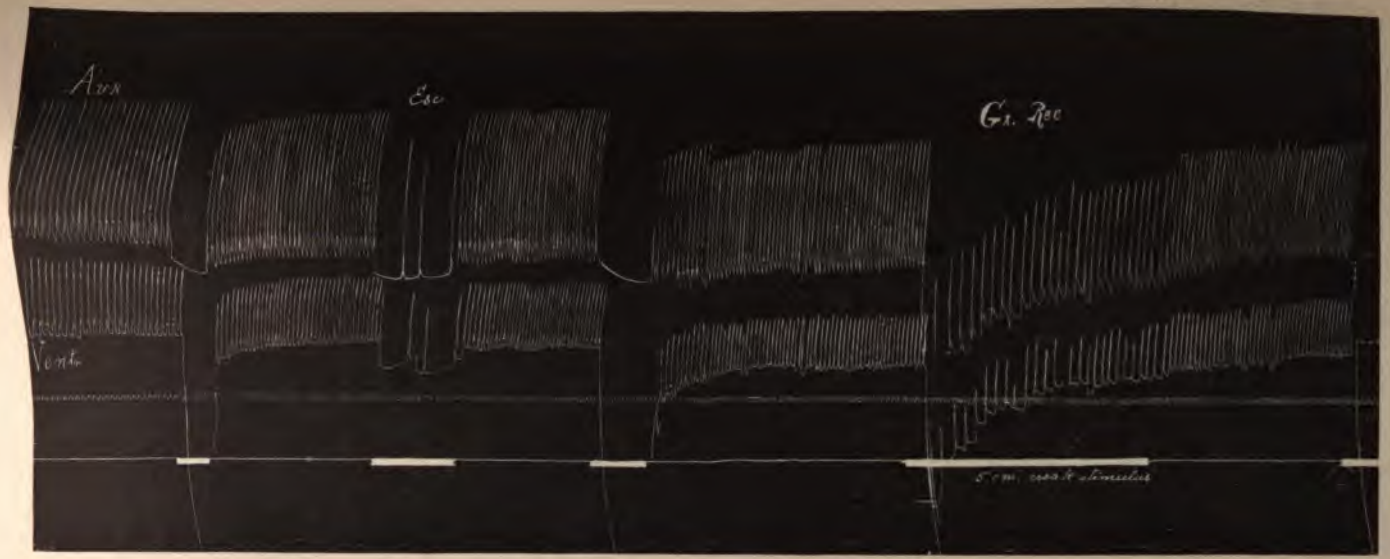
2. Beginning with the secondary coil at zero and the vagus on the stimulating electrode, gradually increase the strength of the current until the threshold is reached. What is the meaning of this term? Its effect is evident from the first beginning of slowing in the rate of the heart contractions.

3. Having determined the threshold, continue to increase the stimulus until the heart beat is arrested entirely.

4. Allow the heart to recover by stopping the stimulus. Note that there is an after-effect. Compare the rate of the heart beat in this after-effect with the normal heart tracing recorded at the beginning of the experiment. Is the rate faster or slower than the normal? Explain the after-effect. In the Elosmobranch fishes there is no demonstrable after-effect (see tracing Fig. 27, p. 23).

5. Stimulate the main trunk of the vagus by a current known to arrest the heart completely. Continue this stimulation for one minute. Note that the arrest does not occur immediately in the second that the signal magnet records the beginning of the stimu-

[1] "Zur Technik von Vagus Experimenter am Herzen von Scyllium Mustelus, Canis, etc." By J. C. Hemmeter, in *Zeitschrift f. biolog. Technik.*, Bd. II, S. 221, 1911




TRACING OF VAGUS INHIBITION TAKEN SIMULTANEOUSLY FROM AURICLE AND VENTRICLE OF THE HEART OF A SHARK. (*Hemmeter.*)

FIG. 27.—After the instant of vagus stimulation (see signal record) there is a latent period of 1–2 beats in the auricle before complete inhibition sets in. The ventricle beats three times more than auricle before it stops, this is an apparent delay in ventricular inhibition brought about by the fact that the ventricle receives its excitation to contraction from the auricle. But the time of downward propagation from auricle to ventricle is so short that this could not be shown in a time record of seconds. It is evident that the auricle is inhibited first and thereafter the ventricle gives 2–3 further contractions, showing that prevailing influence of the chronotropic fibers is on the auricle. The metronome is recording in seconds. The heart of this shark is beating once in a second. Esc = escape from vagus stimulation. Gr. Rec = gradual recovery from inhibition after weak stimuli. The lowest straight line is the line of signal magnet. (*Marine Biol. Lab. of the U. S. Bureau of Fisheries, Woods Hole, Mass.*)

lation. Note that there is a latent period of one or two heart beats. Note that if the stimulation continues, the heart will resume beating even during the stimulation. It is impossible to inhibit the heart for longer than one minute. Study these phenomena on the accompanying tracing.

## Effect of Vagus Stimulation on the Auricular and Ventricular Beats Compared.

Connect both the auricle and ventricle each to a separate heart lever by the suspension method as described (p. 69). Record a clear double tracing, the auricular above the ventricular contraction with a time record in one-half seconds beneath these; traced by the metronome. After good records have been secured, stimulate the main trunk of the vagus with a strength of current just slightly above the threshold valve. Observe that weak stimuli affect only the auricle but that strong stimuli produce inhibition of both the auricle and the ventricle. Note in the tracing (Fig. 27) that ventricle in some animals gives 2 to 3 beats after the auricle stops. Explain. It is said that the vagus contains four kinds of fibers. 1. Those that influence the *rate;* according to Engelmann they are designated as *Chronotropic.* 2. Those that effect the *strength, or inotropic* fibers. 3. Those that effect the *conductivity* or *dromotropic.* 4. Those that effect the *irritability* or *bathmotropic fibres.* The chronotropic fibers extend only to the sinus venosus and auricle, stimulation of the vagus branches below the auriculo-ventricular groove, causes only weakening of the strength, no slowness and inhibition.

The vagus of the frog contains both inhibitory as well as accelerator fibers and stimulation of the mixed nerves usually causes arrest of the beat, but if the nerve is cooled, the inhibitors are paralyzed before the accelerators and upon stimulating a cooled vagus, if the proper temperature is observed, only acceleration should be obtained.

## Inhibition of the Heart by Reflex Stimulation of the Vagus.

GOLTZ'S EXPERIMENT. *Directions.*—Expose the heart of a frog slightly anesthetized by ether. Count the number of heart

beats in a minute. While one student continues counting a second student should tap the abdomen above the stomach with the handle of a scalpel at the rate of two taps per second. The heart is slowed and eventually it will become inhibited. This is due to afferent impulses that first reach the inhibitor center in the medulla and then travel centripetally down the vagi to the heart. Cut both vagi and repeat the tapping and counting. Note that the reflex inhibition will not take place after both vagi are severed. Expose the sciatic nerve of the frog. Secure it with a thin ligature and divide it distally therefrom. Stimulate the distal end. Is there any effect? Stimulate the central end. Note that the heart will be inhibited. Cut both vagi. Stimulate the central end of the sciatic again. What is the effect now? Explain.

## Irritability and Conductivity of the Inhibited Heart.

*Dromotropic and Bathomotropic Effects during the Chronotropic Arrest.*—Produce complete inhibition of the heart of a large slider terrapin by strong stimulation of the vagus. Sharply touch the ventricle with the point of a needle or forceps. Note that it will respond by a single contraction. The heart during inhibition is therefore still irritable. Allow the heart to recover. With a stop-watch determine the time of the contraction made from its beginning in the sinus venosus until it reaches the apex of the ventricle. Produce complete inhibition of the heart once more. Stimulate the sinus venosus at the entrance of the great vein. Again determine by the stop-watch the rate of the contraction made from the sinus to the apex. It will be slower during inhibition than it was normally. This experiment is more accurately carried out by connecting the sinus and also the apex each to a separate heart lever, arranging a signal magnet and a time record in fourths of a second. After obtaining a normal record of sinus and ventricle, complete inhibition of the heart is produced by stimulating the vagus with a strong current. During this stimulation and with a second pair of electrodes that are connected with the signal magnet, the heart is

stimulated by single induction shocks. It can then be graphically recorded during the heart contraction thus made, that the conduction is slower during vagus inhibition than normally.

### The Inhibitory Center.

EXPERIMENT.—Etherize a frog. Place the animal back uppermost on the frog board. Remove the bones from the roof of the skull, clearing the parietal and occipital bones. Also remove the laminæ of the first three vertebræ and expose this part of the spinal cord. Expose the heart by the simpler method described and hold the frog in such a manner that the heart can be seen and its rate counted while the brain, medulla and spinal cord are stimulated. Stimulate the spinal cord with a tetanizing current sufficient to be detected by the tongue. The heart will continue to beat at its normal rate. Stimulate the cerebral hemispheres; the heart will continue at its normal rate. Stimulate the medulla; the heart will be arrested. This arrest can be produced without the previous dissection of the medulla by pushing the needles of the electrode deep into the tissues between the head and spine close to the shoulder blades of the frog.

## CHAPTER VI.

## The Effect of Chemical Substances and Poisons on the Heart.

EXRERIMENT. 1. *Effect of Atropine.*—Having determined the threshold of electric stimulation which would cause slowing of the heart beat, take a normal tracing by the suspension method and a time tracing underneath of this. Stimulate the vagus and note the strength of current sufficient to cause inhibition. Drop three drops of a 1 per cent. solution of atropine sulphate upon the sinus and auricle; wait 3–5 minutes then stimulate with the same intensity of current that was effective before. This stimulus will now be ineffective. The vagus terminations are poisoned or paralyzed by the atropine. Apply a single induction stimulation to the sinus venosus. Is the effect of inhibition produced? Wash the heart thoroughly by applying normal salt solution.

Allow the heart to recover. Apply to the recovered frog heart a 1 per cent. solution of muscarine.

*Effect of Muscarine.*—Soon after applying this substance to the heart a diastolic arrest results, having the character of a vagus inhibition. Wash away the muscarine with normal NaCl solution. Now apply once more the atropine solution. The heart resumes its pulsations.

*Effect of Nicotine.*—When experiment with heart poisons are made it is necessary always to precede the application of the poison by obtaining a normal record of the heart contraction, and following this normal record to obtain a second tracing in the course of which inhibition has been produced by stimulation of the vagus. Study the character of the normal heart tracing and observe the movements of the organ itself. Drop upon the heart a 0.1 solution of nicotine in physiologic salt solution. While a record is being taken, study the rate and the strength of the heart beat. Is there any change? Now stimulate the vagus with a strong tetanizing current. No inhibition will be produced. Now stimulate the junction of sinus venosus with the auricle. Inhibition will be produced. Curare and conine resemble nicotine in this effect. The facts so far observed may be explained as follows:

Nicotine paralyzes the ganglia through which the vagus fibers pass. It does not paralyze the very ends of the vagus in the heart muscle. Stimulation of the sinus which is practically stimulation of the vagus fibers between the ganglion cells and the muscle fibers of the heart is therefore effective although the stimulation of the trunk of the vagus is not. W. H. Gaskell in Schafer's "Handbook of Physiology," Vol. II, page 20, states there are no efferent cells on the course of the vagus until the intrinsic heart ganglia are reached; and Langley found by the nicotine method that each efferent visceral nerve possesses upon its course from the central nervous system to its termination in the peripheral organ or tissue one, and only one, nerve cell. The poisons of the atropia group *paralyze* the nerve endings themselves so that neither stimulation of the sinus nor of the nerve trunk can cause inhibition. So

nicotine and atropine have a *paralyzing* effect. Muscarine, on the contrary, has a *stimulating* effect; it *stimulates* the vagus fibers between the nerve cells and the muscle, or the actual nerve endings, and thereby keeps the heart in a state of permanent inhibition which is removed, as we have seen in the preceding experiment, when atropia eliminates the nerve endings. In some of the elasmobranch fishes one occasionally meets with individuals whose vagus have no, or very little, inhibitive power. This is found in some specimens of the sand shark (Carcharias littoralis) where occasionally one finds a vagus that fails to hold the heart inhibited for more than two or three beats. On such hearts, muscarine has no effect. This is in accordance with the theory just announced.

*Effect of Pilocarpine.*—After taking a normal tracing as before, and a tracing including a vagus inhibition, bathe the heart in a 0.1 per cent. solution of pilocarpine hydrochlorate. Inhibition can now be produced with a weaker stimulus of the vagus than before. Apply five or more drops of pilocarpine solution. The heart will eventually be inhibited. Bathe the heart in normal salt solution; then add a .2 per cent. solution of atropine sulphate. The heart will resume its beat and escape inhibition. These two poisons are antagonistic on the heart just like muscarine and atropine. The causes are the same as with the latter two substances.

The study of these toxic substances on the heart is not done for the sake of demonstrating any pharmacologic or therapeutic principle though these experiments may be valuable in this direction, but they are here introduced to convince the student that chemical unions are possible between nerve cells, ganglion cells, muscle and nerve fibers and various toxins and that the effects of one can be offset by the effects of the other. It is difficult to say what is normal and what is abnormal in the action of poisons on the heart, because the animal body under physiologic conditions creates chemical substances in the thyroid gland and in the adrenal and pituitary bodies which have a very decided influence upon the heart structures. In the intact organism

they act as normal influences, but in an experiment it is almost impossible to preserve normal conditions.

*Effect of Thyroiodine.*—Connect the frog's heart with the heart lever by the suspension method. Draw records before, during, and after vagus stimulation. Determine the threshold at which the minimal stimulus is just effective in slowing the heart. Drop a 1 per cent. solution of Merck's thyro-iodine upon the heart. Determine the threshold again. It will require less intensity of current to produce slowing. According to Cyon, thyroiodine increases the sensitiveness of the terminal end apparatus of the vagus. In some diseases the thyroid gland is gravely disorganized and, according to the stage of the disease, too much or too little thyroiodine may be produced and as a result we may have either excessively fast or unreasonably slow heart rates. The effect of some of the special heart toxins cannot be studied by attaching the ventricle to the heart lever, but an adequate understanding of their effect necessitates that an artery should be connected by a cannula with a mercury manometer. The effects of digitalin and of adrenalin and extract of pituitary gland can only be correctly studied in this manner. Dropping these substances in solution directly upon the heart while tracings are being taken as before, may indicate a strengthening of the heart beat when the heart is nearly exhausted. A saturated solution of digitalin should be used and a solution of adrenalin chloride in physiologic salt solution in the proportion of 1 to 10,000. Note whether there is any effect on the force and rate of the heart systole and whether the rate of conduction is plolonged or retarded. Then, too, the effect of very small doses and very excessive doses requires experience and very careful measurement, for they are occasionally diagonally opposed to each other.

*Effect of Adrenalin on the Size of the Blood-vessel.*—In the frog's mesentery, lung, or preferably the web of the foot, locate under the microscope a certain vessel with very distinct outlines. Add a few drops of the above adrenalin solution and observe the effect on the dimensions of the vessel and rate of current, with the micrometer.

## The Inner Stimulus of the Heart.

Review lecture notes on electrolytic dissociation, electrolytes, and non-electrolytes. Read the chapters on the subject in Jacques Loeb's "Dynamics of Living Matter," especially his distinction between a nutrient solution and a chemically and electrolytically balanced solution. (see Appendix.)

Many years ago Ringer showed that contractile tissues continued to manifest their activity in certain saline solutions. The solution best suited has been named after him and Ringer's solution consists of the chlorides of potassium, sodium and calcium, together with a very small amount of sodium bicarbonate in the following proportion:

Ringer's Solution.

| | |
|---|---|
| Sodium chloride, | 0.9 per cent. NaCl |
| Potassium chloride, | 0.042 per cent. KCl |
| Calcium chloride, | 0.024 per cent. $CaCl_2$ |
| Sodium bicarbonate, | 0.02 per cent. $NaHCO_3$. |

If glucose is added to this in the amount of 0.1 to 0.2 per cent. it will represent the solution recommended by Locke.

Loeb not only expanded the views of Ringer but he interpreted the effects observed as due to ionic action. Contractile tissues will not contract in solutions of non-electrolytes, such as sugar, urea or albumen, but different contractile tissues vary in the nature of the kind of ions which are the most favorable stimuli for them. The heart muscle of various animals also differ, not only in the kind of ions, but also with regard to the proportion of these ions that must be present in individual cases. W. H. Howell considers that the ions represented in Ringer's solution, which are those of the normal plasma, are important factors in the causation of the heart beat and therefore he considers them as the factors of the inner stimulus. The hearts of Elasmobranch fishes cannot be kept alive on Ringer's solution. They require the presence of 20 grams of urea and 20 grams of NaCl to 1000 c.c. $H_2O$. (Baglioni—Hemmeter.)

EXPERIMENT.—The hearts may either be used from the turtle or the frog and at first the phenomena should be studied by strips cut out of the ventricle of the turtle, and later an intact heart may be excised, connected to a bent glass rod, which is immersed in the solution of the electrolyte to be studied. (See illustration, Fig. 28, p. 83.)

When a strip of the ventricle is excised it should be attached to the light muscle lever by one end, and the other end to a glass rod bent at right angles in such a way that it can be immersed in a small beaker. The upper end of the glass rod is held stationary by a clamp. The lever is adjusted to a slowly revolving kymograph. These muscle-strip preparations from the heart occasionally cease beating before the preparation and apparatus is arranged. Then it can be readily studied whether the immersion in one or the other of these electrolytes will restore the beat.

*Effect of Sodium Chloride, Especially the Kations of NaCl.*—1. Immerse the preparation in a beaker containing 0.7 per cent. solution of NaCl. Record the contractions on the drum. 2. Compare the rate and character of the contractions with those previous to the immersion, if any. How long before the contractions will cease? 3. After they have ceased, blot off the excess of NaCl with filter-paper and immerse the preparation in isotonic solution of calcium chloride about 1 per cent. Do contractions occur? if not, reimmerse in the sodium chloride solution. Is an isotonic solution of NaCl alone sufficient to maintain the heart contraction?

*Effect of Calcium Chloride and Its Ions.*—Immerse another strip of heart muscle in a 1 per cent. solution of 2($CaCl_2$). Compare the record with that obtained by NaCl. Are the contractions stronger or weaker? When the contractions have eventually ceased, what is the state of the heart muscle? What is calcium rigor? What is meant by potassium inhibition?

## Simultaneous Action of Sodium and Calcium.

To a physiologic NaCl solution add 0.1 of its volume of a 1 per cent. ($CaCl_2$)2 solution. Immerse a fresh heart-muscle

preparation. Compare the time in which the muscle will beat in this with the time it maintained its contraction in pure NaCl and in pure $CaCl_2$ alone.

*Effect of Potassium Ions.*—After a strip of heart muscle has been beating well in a solution of NaCl and $CaCl_2$, immerse it in a solution of potassium chloride 0.9 per cent. which is isotonic with a 0.7 of NaCl. The contractions will cease.

*Effect of Combined Actions of Sodium, Potassium and Calcium.*—Prepare a Ringer's solution according to above formula (p. 80). Immerse a strip of ventricular muscle attached to a writing lever. Record the contractions on a slowly moving drum. Compare the time in which the heart muscle will contract in Ringer's solution with the time it contracted in a solution of each of the constituents alone. With some hearts it is impossible in the laboratory hours to await the cessation of the contraction in Ringer's solution; the heart of the spider crab has been recorded in contraction for twenty-four hours in solutions of various electrolytes.[1] Howell is of the opinion that the effect of the vagus nerve on the heart is due to the setting free of potassium ions in the coronary blood stream from some easily dissociable compound of potassium in the heart muscle.

## The Contact Irritability of Jacques Loeb.

During the observation of the white corpuscles in the circulation of a frog's web, the student will have observed that the moving white corpuscle is guided by the contact with red corpuscles or the vessel wall or the chemical substances produced by the presence of the irritant or bacteria. The chemical character of the body with which leucocytes come in touch also determines whether or not they give fibrin ferment and cause coagulation of the blood, or other liquids that contain fibrinogen.

1. Certain salt solutions (1 gram-molecule in 8 or 10 liters) bring about an apparently new form of irritability in muscles, which may be called provisionally contact irritability. A muscle that has been treated in this way will contract powerfully

[1] Experiment by my assistant, Dr. A. C. Carroll, in the Marine Biologic Laboratory, Woods Hall, Mass.

when it passes from the salt solution to air, $CO_2$, oil, sugar solutions, etc., or from glycerine solutions, sugar solutions to air.

2. The salts whose solutions produce this form of irritability are (with one exception) sodium salts, whose anions are capable of precipitating calcium, namely:

| | | |
|---|---|---|
| Sodium fluoride | $Na_2HPO_4$ | Sodium citrate |
| Sodium carbonate | Sodium oxalate | Sodium tartrate |

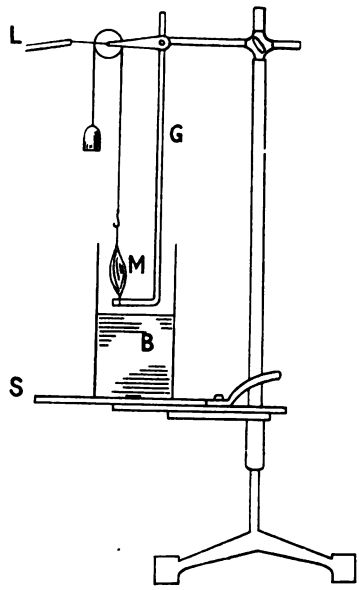



FIG. 28.—Apparatus for demonstrating Loeb's contact irritability. *L*, Lever, *M*, Muscle; *G*, glass rod; *B*, beaker containing solution; *S*, shelf capable of being raised or lowered.

3. If the nerve alone (without the muscle) be put into one of these salt solutions (1 gram-molecule in 8 or 10 liters) the muscle begins to twitch in about five minutes and finally goes into tetanus. If the nerve be taken out of the solution the contraction ceases. Although this seems to indicate that the salts or their ions stimulate the nerve directly it can be shown that they only modify or increase the irritability of the nerve. For when the same nerve

is brought into contact with any solid or liquid body (conductor or non-conductor) the contractions of the muscle will be resumed while they will gradually cease or diminish when the nerve is again surrounded by air on all sides.

4. The fact that certain ions are capable of bringing about forms of irritability in nerves and muscles which do not exist normally may perhaps furnish the explanation of a number of certain morbid phenomena (neuroses, hysteria) in which the motor and sensory reactions of the patient are modified.

Transfer a muscle preparation arranged as described:

Contractions occur when the muscle passes:

| From | To |
|---|---|
| Sodium citrate solutions | Air |
| Sodium fluoride solutions | $CO_2$ |
| Sodium oxalate solutions | Oil |
| Sodium carbonate solutions | 2 n sugar solution |
| Etc. (see above). | Glycerine |
| | Chloroform |
| | Toluol |
| | Mercury |

Relaxation of the contracted muscle will occur when the muscle passes from any medium in the right column above to any medium in the left column.

After the muscle has been treated for some time with any of the efficient solutions (Na-citrate, etc.) the contractions are also produced when the muscle passes:

From n/8 or n/4 sugar solution to air.
From n/8 or n/4 glycerine to air.
From *any* salt solution to air.

A very interesting and theoretically important fact is that the muscle loses this peculiar form of irritability very soon when it remains in contact with air, oil, sugar solution, glycerine, or salt solutions different from those that produce this specific irritability. In LiCl or NaCl solutions the contact irritability is lost as fast, if not faster than in a sugar or glycerine solution. We can re-

establish the irritability, however, if we put the muscle back into the sodium citrate solution for some time. This fact, together with those mentioned before, suggests the following as the most probable explanation of the peculiar phenomena of contraction with which we have been dealing; the solutions which produce the contact irritability possess anions that are liable to form insoluble calcium compounds. They are all with one exception—namely $(NH_4)_2SO_4$—sodium salts. Whatever the effects of these anions may be, the fact that in less than a minute the contact effects are noticeable indicates that only the surface layer of the muscle or what is less probable, the surface layer of each individual, fiber, is altered. It is impossible for the anions to migrate deeper into the muscle in so short a time. I regard the phenomena as partially due to altered surface tension.

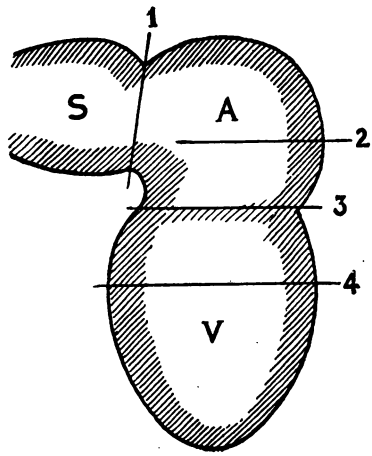



FIG. 29.—Schematic frog's heart, to show application of the Stannius ligatures: 1, Between sinus (*S*) and auricles (*A*); 2, middle of auricles; 3, between auricle (*A*) and ventricle (*V*), at auriculo-ventricular groove; 4, about base of ventricle, below groove. (*Busch.*)

**Stannius's Experiment.**—Pith a frog and expose the heart.

1. *First Ligature.*—Tie the frenum, the partition of pericardium attached to the dorsal aspect of the ventricle, and use the ligature as a guide. Pass a thread around the junction of the sinus with the auricles and tie snugly. The sinus continues to beat. The auricles and ventricles stop beating.

2. *Second Ligature.*—Now tie a second ligature around the heart at the auriculo-ventricular groove. The ventricle again begins to beat. The auricles will probably remain quiescent.

3. *Third Ligature.*—Tie a third ligature about the middle of the auricles, as indicated in the figure. The auricles will again begin to beat, but at a different rate from that of the ventricle.

4. *Fourth Ligature.*—Now tie a fourth ligature about the base of the ventricle. The ventricle will again cease to beat.

## Interpretation of the Stannius Experiment.

An extensive literature has developed about the physiologic meaning of this simple experiment which now, however, has chiefly an historic interest only (see Löwit, Pflüger's Archiv., 23, p. 313, 1880; also F. B. Hofman, in Nagel's Handb. d. Physiol. d. Menschen," B. I., p. 224). It is a fact of comparative physiology that the entire heart beats with the rhythm of that part of it that possesses the maximal number of contractions per minute. This means that the coordination of the heart is effected by the influence or tendency of the sinus venosus which contracts most frequently, to force the remaining parts to beat in the same rhythm (Jacques Loeb, Einleitung in d. Vergl. Gehirnphysiol). Therefore as the excitation to heart contractions has its origin in the sinus venosus, anything that severs the connection of the auricle and ventricle from this sinus would bring them to a standstill (Eckhard, Beitr., 1, 147, 1858, in Müller's Archiv., also 1852, p. 163). But as the parts that are cut off begin to beat again after a time, Heidenhain assumed that automaticity was a property of all parts of the heart in the same degree and accordingly he explained the arrest of auricle and ventricle after the first ligature by assuming that the intracardial inhibitory nerves are strongly stimulated thereby and the contractions of the auricle and ventricle transiently suppressed. One interpretation of the first ligature is that the auricle and ventricle need some time for the development of their own intrinsic automaticity to the point at which an independent rhythm can originate. The effects following the

second ligature are in the light of this interpretation supposed to be due to stimulation of the muscular tissue in the auriculo-ventricular groove by the ligature.

The second interpretation (Haidenhain) is a purely neurogenic one, according to which the inhibition is due to stimulations of vagus fibers at the junction of sinus and right auricle by first ligature. The second ligature cuts off the ventricle from these inhibitory impulses, but still leaves the auricle under their influence (*v.* Cyon, Die Nerven des Herzens).

The truth may lie midway between these two interpretations, *i.e.*, auricle and ventricle do receive their impulse from the sinus and hence any cut off impairs their coordination; but at the same time it is unavoidable to stimulate the vagus also by the first ligature.

In the Elasmobranch fishes it is impossible to inhibit the heart ventricle or auricle by a first ligature around the junction of sinus and auricle and the ventricle beats at a more rapid rate after the first ligature (Hemmeter). In mammals the Stannius experiment fails.

## Ventricular Contraction. Direct Transmission Method of Recording.

*Apparatus.*—Pithing needle, frog, bowl, scissors, knives, forceps, glass rod, frog board, glass plate, towels, normal saline solution wires, silk ligatures, aneurysm needle, heart lever, stand, wooden stool, heart holder, recording apparatus, dry cells, inductorium, wires, simple key, ligature, signal magnet, clamp, stand.

**Heart Holder.**—The heart of the animal, frog or small terrapin, is placed on *C*; then *D* is brought lightly to rest on the upper surface of the ventricle. A long light straw tipped with a fine point of tin foil is inserted through *L* and the tin-foil tip brought in contact with the kymograph.

The heart may be electrically stimulated through *L* from the binding posts *A* and *B*..

*Directions.*—Fasten the brainless frog down on the frog-board. Only destroy the brain. Expose the heart by Njegotin's method. Set the heart holder across the frog board. Raise the heart gently with seeker and pass spoon-like tongue of the holder beneath the heart. Fill the spoon with NaCl solution, rest the upright levef

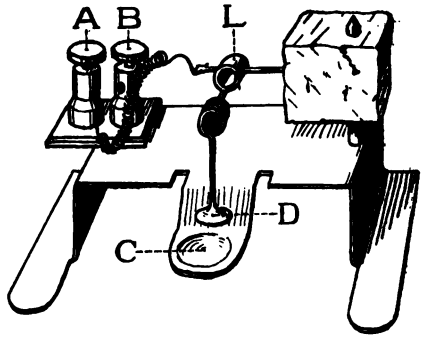



FIG. 30.—Heart holder. (*J. C. Hemmeter.*)

on the ventricle, but do not allow the weight of the lever to remain on the heart too long. Adjust lever on a slow moving drum.

### Staircase Contractions ("Treppe" *of the Germans*).

Prepare a frog's heart as in the preceding experiments. Connect the apex of the ventricle with the heart lever either by the suspension or the direct method. Ascertain the *threshold* or least intensity of stimulus still capable of causing a contraction. If desired arrange a time record beneath the heart tracing and turn the drum by hand unless an automatic kymograph regulator (Zimmermann, Leipzig) is obtainable which permits this movement to be executed by the apparatus. Repeat the stimulus every five seconds. This can also be done automatically in case the proper apparatus is at hand, otherwise by the simple key and a stop-watch. The ordinates of the ventricular contraction records will rise one above the other so that each apex of ventricular curve is a little higher than the other, but a line touching and joining the

apices will form an hyperbola. With the frog-board myograph already described, the curarized gastrocnemius muscle will give successively stronger contractions to repeated stimuli of uniform intensity. This may be interpreted as meaning that the irritability of muscle is increased by chemic products formed during contraction. For the identical stimulus effects a greater contraction when it succeeds a brief tetanus than it did before. (Bohr and Rosebach.)

## Maximal Response of Heart Muscle to Minimal Stimulus.

Stop the rhythmical contraction of a frog's heart by applying the first Stannius ligature.

Set up the inductorium for single induction shocks. Connect the tip of the ventricle with the cardiograph lever. Arrange the drum for movement by hand. With the secondary coil removed as far as possible from the primary, apply the electrodes to the ventrical and break the primary circuit. No contraction will probably occur.

Move the secondary nearer the primary and repeat the breaking of the circuit, at intervals of ten seconds, until a stimulus is found which will cause the ventricle to contract.

Move the drum slightly, increase the strength of stimulus, and record again. Repeat with stronger and stronger stimuli.

The contraction in response to the strongest stimulus is no greater than the one in response to the weakest stimulus that will cause a contraction. The heart will not give "submaximal" contractions.[1]

Compare this with the response of skeletal muscle to stimuli of various strengths.

**Refactory Period.**—Adjust the frog's heart in the spoon-shaped holder as described before for the direct transmission method. Record the contractions upon the medium rapid drum. Arrange a signal magnet in the primary circuit. Be careful to adjust the point of its writing lever directly under the point of the lever extending from the heart holder. Now record a normal heart tracing.

[1] This phenomena is seriously questioned in recently published experiments.

Stimulate the ventricle at intervals of ten or fifteen seconds with maximal make or break induction stimuli. Each time the stimulus should be applied at a different part of the heart's cycle.

At certain stimuli there will be an extra contraction; others will have no effect so far as calling forth an extra contraction is con-

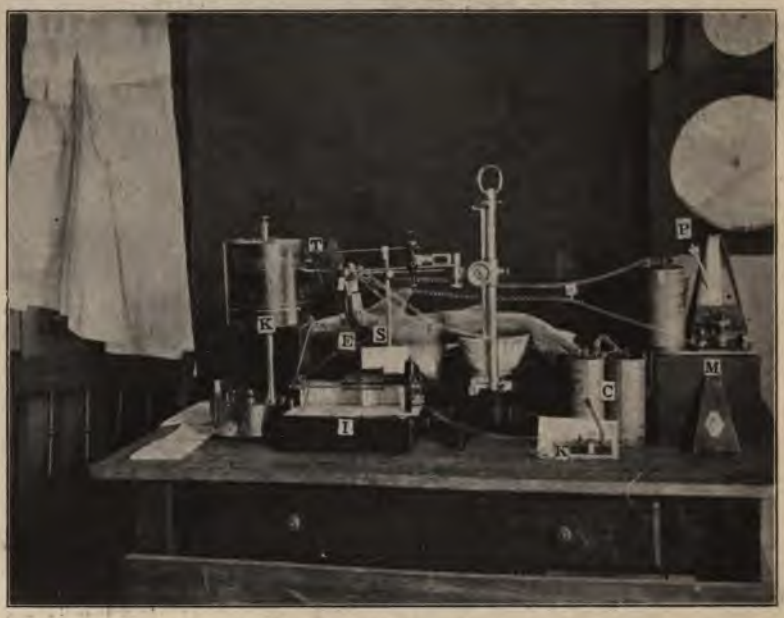



FIG. 31.—Arrangement of apparatus for effect of vagus stimulations on the heart and also for study of the refractory period. K, kymograph, the smoked paper showing heart contraction wave, signal magnet line and time tracing; S, animal (in this case a small shark) (photo was taken at the Biol. Laboratory of the U. S. Bureau of Fisherie's at Woods Hole Mass.); K, simple key; C, cells; M, metronome; P, pendulum of metrotnome—wires from this go to time recorder T. The large metal upright with top handle is the universal stative the extensions of which hold writing pen, signal magnet and time recorder.

cerned. The first stimuli were applied during the *irritable* stage of the heart muscle. What period of the heart cycle is this? The second stimuli were applied during its *refactory period.* What period of the cycle does this correspond to?

Where an extra contraction occurs, attempt to record the pause rhythm which prevailed before the extra contraction was produced.

## CHAPTER VII.

1. *The number of the red and white corpuscles.*

2. *Changes of size and chemic composition of red blood-corpuscles and of concentration of the plasma during pulmonary and tissue respiration.*

*Apparatus.*—Hemocytometer, pipette, soap, water, $H_2O_2$, alcohol, ether, sterile needle, microscope, test-tubes, barometer tubes.

### The Counting of the Erythrocytes or Red Corpuscles.

The instrument most generally used is the Thoma-Zeiss hemocytometer. It consists of two parts: a mixing pipette and a counting stage. The pipette (Fig. 32) is a capillary tube graduated in tenths with an expanded bulb of exactly 100 times the capacity of the tube from the tip to the mark 1. In one case blood is drawn to the mark 1 and then a diluting fluid drawn to the mark 101 at the upper end of the bulb, the bulb containing the fluid diluted 100 times. In the other case the blood may be drawn to the mark 0.5 and the diluting fluid as before, the blood being diluted 200 times.

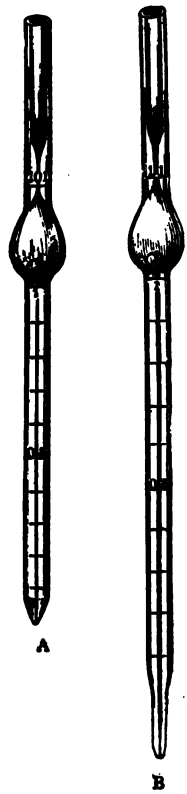



FIG. 32.—Diluting pipets; A, Erythrocytometer; B, leucocytometer.

The fluid used for diluting may be Toison's or Hayem's, or normal salt solution may be used. The bulb contains a glass bead to facilitate mixing the blood. This should not stick to the walls before drawing the blood.

The other part of the instrument is the micrometer slide upon which the diluted blood is evenly spread for counting the corpuscles. This consists of a glass slide, Fig. 33, upon which is mounted a covered disk, a square millimeter of which is subdivided by a dividing engine into 400 squares.

The 1/20 mm. micrometer, is surrounded by an annular cell, 8, the sides of which project one-tenth milimeter above the surface. This cell is closed by a thin, flat cover so that the cubic space included between each small square of the micrometer and the cover would be 1/4000 of a cubic milimeter.

The blood to be examined is diluted 100 or 200 times, preferably 200 times. Therefore the number of corpuscles that each little compartment holds is that number which has been diluted 200 times or $(1/400 \times 1/200) = 1/800{,}000$ cu. mm. of blood. We


FIG. 33.—Thoma-Zeiss counting chamber.

find the average number of corpuscles to each small square and multiply that number by 800,000 to obtain the number of corpuscles per cu. cm.

*The Practical Method.*—Aseptically cleanse the tip of the finger or, preferably, the lobe of the ear, with soap and water. Wipe the ear with a cloth wet with alcohol. Dry thoroughly. With a sterilized needle or a sharp pen with one nib broken off, make a quick stab of the ear. Wipe off the first drop of blood, for this clots more readily than that which oozes later. Blood should ooze freely from the puncture without pressure. Pressure disturbs the normal make-up of the blood. Insert the point of the pipette well into the blood drop and carefully draw in blood to the 0.5 mark on the stem of the pipette. With a cotton cloth wipe off all blood adhering to the outside of the pipette. Dip the end of the pipette into the diluting fluid and draw this in through the stem and into the ball until the 101 mark is reached. The pipette should be closed at both ends with the thumb and

forefinger and shaken well in order to obtain a uniform distribution of the corpuscles throughout the mixture.

*The Filling of the Counting Cell.*—The counting stage must be clean. If it is clean it will show Newton's rainbow rings when the cover-glass is pressed against the green glass by means of a pin. After blowing out a few drops from the pipette a small drop is transferred to the glass disk. No dust must be between the green glass and cover slip nor must the drop be so large that

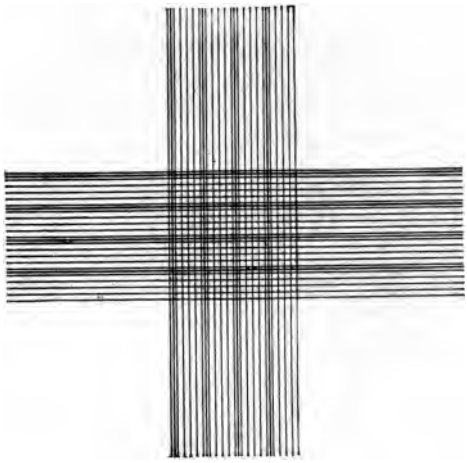


FIG. 34.—Ruled surface of Thoma-Zeiss counting chamber. (*Da Costa.*)

any part of it runs between these two glasses, otherwise the cover slip will be raised more than 1/10 mm. above the disk.

Wait several minutes for the corpuscles to sink to the bottom of the cell upon the ruled squares. It is obvious that the counting cell must be kept in the horizontal position. The corpuscles in each of the 16 small squares are separately counted. In the count of each square are included the corpuscles lying upon the left and upper lines. Begin with the square in the upper left hand corner, counting all the cells within the space, also those upon the left and upper lines and in like manner run across each of the four squares, then the four squares in the second row and so on until 16 squares are counted. To make an accurate count, 128 small squares should be counted, *i.e.*, the 16 squares in 8 of

the large squares. Then another drop is used and another 128 squares counted. The average number of corpuscles in one small square is then multiplied by 800,000. After completing the estimation the pipette must be cleaned by reversing the rubber and drawing out the contents first with distilled water, then with alcohol and finally with ether and air. The counting stage is to be cleaned with *water alone*. Alcohol and ether may, by dissolving the cement, ruin the apparatus.

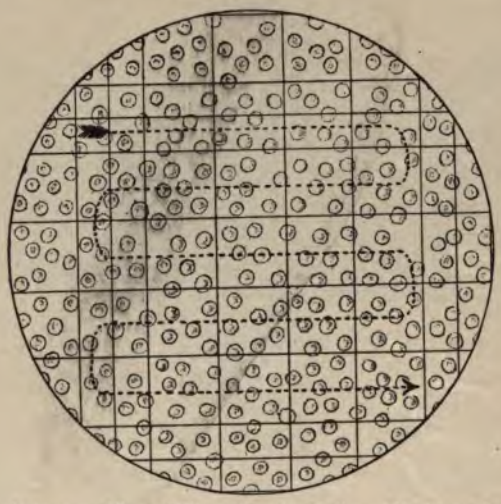


FIG. 35.—Plan of Counting the Cells. (*Da Costa.*)
The small squares are examined in the order indicated by the arrow.

*Cleaning of the Cell and Pipette.*—Carefully rinse the cell with distilled water and dry with a soft cloth. Treat the cover in the same manner. Do not use alcohol or ether because they will coagulate the albumin of the blood.

In cleaning the pipette first blow out any of the diluted blood remaining. Fill with distilled water several times. If all traces of blood are not removed, wash out with hydrogen dioxide and follow this with a washing out with distilled water again. Draw alcohol through by suction and follow this with ether, drawing through a stream of air until the pipette is thoroughly dry. This is shown by the bead enclosed in the bulb not adhering to the sides.

## Counting of the Leucocytes or White Blood-corpuscles.

Because of the smaller number of the leucocytes than of red blood-corpuscles, the dilution required is much less. A different mixing pipette is used, one that dilutes the blood 10 to 20 times. The diluting fluid is 3 per cent. acetic acid. This serves the double purpose of disintegrating the red blood-corpuscles and accentuating the nuclei of the white corpuscles. The blood is drawn up to the mark 0.5 and then the acetic acid to the mark 101, thereby diluting the blood 20 times. Proceed as before with this exception. In counting, the white cells are counted in *every large* cell. By including the triple lines, which are simply three large single lines drawn equidistant, there are (20×20) 400 small squares ruled in the disk. Count all the whites within all of the squares, including those in the triple lines. The total number is divided by 400 to obtain the average in one small square and then multiplied by 80,000, (not by 800,000,) since in this case the blood was only diluted one-tenth as much as in case of the red corpuscles:

$\frac{x}{400} \times 80.000 = x \times 200$ is the number of leucocytes in a cu. mm.

As there are 400 squares the calculation in practice is made as follows, to obtain the number of leuccocytes in one cubic millimeter of blood. Normally, for examp' there are 60–70 leucocytes in 400 squares. Say the dilution .s 1 to 10 (one to ten),

$$\frac{60 \times \frac{4000}{400} \times 10}{} = 6000 \text{ leucocytes.}$$

One really only has to multiply the number of leucocytes in 400 squares by 100 in case of a dilution of 1 to 10, or by 200 in case of dilution of 1 to 20, to arrive at the number of colorless blood cells in one cubic millimeter. The most practical solution for dilution is that recommended by Türck:

℞. Acid acetic glacial, 3.0
1% aqueous sol. of gentian violet, 3.0
Aquæ destillat., 300.

## Changes in Size and Chemic Composition of Red Blood-Corpuscles and of Concentration of the Plasma During Pulmonary and also Tissue Respiration.

When blood is saturated with carbon dioxide and then separated into serum and clot, the serum is found to yield more gas than the clot; but if the serum and clot are separately saturated, the latter takes up more carbon dioxide than the former. From this, it is argued that a substance combined with carbon dioxide must in blood saturated with the gas pass out of the corpuscles into the serum. This cannot be hemoglobin, for it remains in the corpuscles, but it may very well be an alkali, combined with the carbon dioxide and thus set free from its connection with the hemoglobin. And, as a matter of fact, under the circumstances described, it has been found that alkalies do pass from the clot into the serum (Zuntz), and chlorine from the serum into the corpuscles (Lehmann), which at the same time gain water and become larger. The molecular concentration of the serum of defibrinated blood, as measured by the lowering of the freezing-point, increases when it is saturated with carbon dioxide. On the other hand, when blood is saturated with oxygen, alkalies pass out of the serum into the corpuscles, which at the same time lose water and shrink in volume, while the molecular concentration of the serum is diminished. Hamburger (Osmotischer Druck u Ionenlehre) has extended these observations to the circulating blood, and has shown that the plasma of venous blood has a higher percentage of alkali, proteid, sugar and fat than the plasma of arterial blood, and that the corpuscles have a greater volume, though not a greater diameter.

When in the *alveoli of the lungs* the red blood-corpuscle takes up oxygen, diminishes in size, takes in an alkali and parts with $H_2O$ because when O combines with Hb to form oxyhemoglobin, this oxyhemoglobin performs slightly the function of a weak acid, neutralizing, as it were, the already existing alkali in the contents of the corpuscle, making its cytoplasm hypotonic, less concentrated, to the plasma of the blood. Now begins osmosis of $H_2O$

from the cytoplasm to the plasma and dialysis of an alkali' probably sodium carbonate, from plasma to cytoplasm. The extent, length of time and degree of osmosis and dialysis depend on the degree of hypotonicity of corpuscles.

When in the capillaries of the systemic vascular system, the red blood-corpuscle takes up $CO_2$ and becomes larger; it takes in $H_2O$ and parts with an alkali.

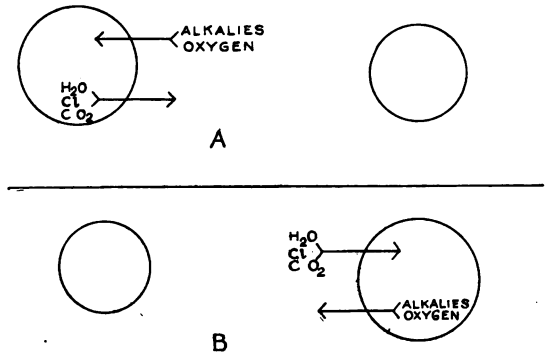



FIG. 36.—*A*, In the lungs the red corpuscle shrinks; *B*, In the systemic capillaries the corpuscle enlarges.

The $CO_2$ made by metabolism of muscle, etc., is taken up by the plasma, the greater part combining with Na to form $Na_2CO_3$ or $NaHCO_3$. The part of $CO_2$ that combines with the alkali in the corpuscle is greater in proportion than that which combines with the alkali in the plasma. This makes the cytoplasm of the corpuscle hypertonic to the plasma. (Now it seems probable that this hypertonicity may be also slightly produced by the release of O from Hb, the neutralizing function of HbO not being exercised.)

Because of this hypertonicity, osmosis of $H_2O$ from plasma to cytoplasm, and dialysis of an alkali from cytoplasm to plasma begins. This action continues until the corpuscle becomes isotonic with the plasma, or nearly so.

The corpuscle is made larger because osmosis exceeds dialysis.

During great muscular exertion, when more $CO_2$ is produced

than usual, the hypertonicity of the cytoplasm is increased. Therefore the amount of $H_2O$ taken up by the corpuscle is greater and the plasma thereby made denser, or more concentrated.

## Influence of Acids and Alkalies on Blood Cells and Plasma.

*Blood-corpuscles.*

The diameter of the red blood-corpuscles diminishes as their percentage of $CO_2$ increases. When the $CO_2$ is replaced by O, the diameter increases again. But diameter and volume do not increase or diminish in a corresponding manner.

Under the influence of five volumes per cent. of $CO_2$, *i.e.*, just exactly the difference in $CO_2$ content between arterial and venous blood, a distinct difference in the volume of red blood-corpuscles can be ascertained.

EXPERIMENT.—Secure 400 c.c. horse blood from a veterinary; treat 100 c.c. of this by bubbling $CO_2$ gas through it from a $CO_2$ generator for one hour. Sediment this blood in a centrifuge. Compare the sediment with that from 100 c.c of normal horse's blood that has not been treated with $CO_2$ but simply shaken with air or oxygen. (Horse blood—sedimented after five volumes per cent. have been replaced by $CO_2$—gives more sediment than the same quantity of horse blood treated with O. For the same reason the volume of blood-corpuscles is larger in blood from the jugular vein than in blood from the carotid artery.

When erythrocytes increase in volume, they always undergo a reduction in their long diameter, they lose their bi-concave form and tend to assume a spherical form.

The red blood cell consists of a semi-solid scaffolding (protoplasm) in the meshes of which the intercellular liquid, the paraplasm, is distributed. The protoplasm takes no part in attracting $H_2O$. Swelling by hypotonic and shrinking by hypertonic solutions are caused by the varying states of the liquid paraplasm.

A diminution in the diameter of the red corpuscles under the influence of the $CO_2$ was described by Manassein.[1] Hamburger first showed that microscopic measurements of the diameter

[1] Berlin, 1872.

could lead to erroneous impressions that the volume of the red corpuscle became smaller by receiving $CO_2$ whereas volumetric determinations proved that by tending to the spherical form and losing their biconcavity they actually increased in volume though their diameter diminished.

*Serum.*

Under the influence of $CO_2$, the serum changes as follows:

1. Alkalinity of the serum increases by the freeing of hydroxyl ions.

2. The serum loses water which passes into the corpuscles.

3. The alkalies of the serum, which before were difficultly diffusible, now become easily diffusible.

4. Loss of pigment, Hb, from red corpuscles occurs in a more concentrated NaCl solution than originally.

## Experiment Demonstrating the Change in Volume of the Red Blood-corpuscles in Venous and Arterial Blood.

Draw from the carotid artery and jugular vein of the dog (whenever possible it is best to use horse's blood, because of its slowness in coagulating) two specimens of blood, say 5 c.c. each. Count the number of red blood-corpuscles in each.

Place the specimens in two small glass barometer tubes, and place the tubes in ice. The object of placing the tubes in ice is to delay the clotting of the blood.

After time enough has elapsed for the blood-corpuscles to settle to the bottom of the glass tubes, the layers of the two sets of corpuscles will be found to be unequal. The red blood-corpuscles (of course there are white ones intermingled) will be found to occupy a larger space in the tube of venous blood than in that containing arterial blood. This cannot be due to a greater number of corpuscles, for it has been determined before that there is an equal number in each tube. Therefore, the reason must be that the cells are of greater volume in the venous blood.

## Blood Pressure—Artificial Circulation Scheme.

*Apparatus.*—Mercury manometer, blood pressure apparatus, plethysmograph, forceps, tambours, bandage, rubber bulb, mercury manometers, kymograph and accessories, thistle tube, rubber membrane with collar button, artificial circulation scheme.

## Estimation of Human Blood Pressure.

The immediate cause of the movement of the blood from the beginning of the aorta through the arteries, the capillaries, the veins to the right side of the heart, is a difference of pressure between these points.

To the positive pressure that causes the blood to flow from the aorta to the vena cava and that causes it to flow from the pulmonary arteries to the veins we give the term *blood pressure*, and may be defined as the pressure exerted radially or laterally by the moving blood-stream against the sides of the vessels.

The causes of the blood pressure are the pumping action of the heart and the compression which the blood encounters in flowing through the arteries. The primary factor in the production of the pressure is the pumping action of the heart.

The secondary factor is the friction between the blood-stream and the sides of the vessels. As a consequence of the friction throughout the entire vascular apparatus the blood experiences a resistance to its onward movement which, working backward, cause the blood to exert a lateral or radial pressure against the walls of the vessels.

The arterial blood pressure may be increased by:

1. Increased rate and force of the heart's contraction.
2. Increased peripheral resistance.
3. Increased general volume of the blood.

And decreased by:

1. Decreased rate and force of the heart's contractions.

2. Decreased peripheral resistance.
3. Decreased general volume of the blood.

*Sphygmomanometry.*—The principle consists in the estimation of the amount of air pressure required to obliterate pulsation of an artery. The instrument used in the clinic and laboratory to estimate the brachial blood pressure (Flaught's) consists of a hollow rubber bag encircling the arm to obliterate the pulsation, a metal pump to supply the air pressure, and a U-shaped mercury manometer to indicate in millimeters of mercury the pressure necessary to overcome the force of the blood current.

It is contained in a case measuring 4 ×4 1/2 ×16 inches.

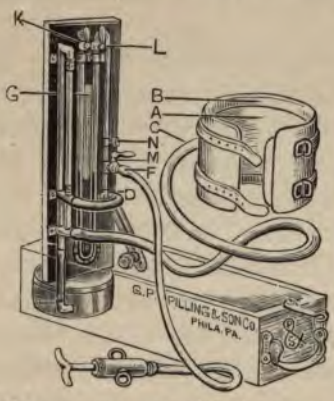



FIG. 37.—Mercury manometer blood pressure apparatus.

The lid is hinged near one end, so that when raised it supports the working parts of the apparatus. A link brace joins the lid and box in such a way that the apparatus may be held in a vertical position, where it is automatically locked during observations.

The U-tube, *H*, Fig. 37, is provided with a scale, *G*, arranged to give the reading directly in Mm. Hg. Loss of mercury from the manometer when the instrument is not in use is prevented by keeping closed two small stop-cocks *K* and *L*, placed upon each extremity of the U-tube.

By a special arrangement, it is impossible to close the lid of the apparatus unless the two cocks above mentioned have been

closed. The scale is adjustable so that if accidental loss of mercury alters the height of the column, it may immediately be corrected and the scales adjusted to zero.

The inner arm-band, *A*, of soft rubber, serves for compression of the brachial artery and it has a reenforced rubber tube, *C*, joining the apparatus at the nipple *D*.

The piston pump furnishes the source of pressure and is connected to the nipple "*L*," bearing a stop-cock "*M*," the function of which is to eliminate elastic pressure of the pump and tube during the diastolic reading.

A needle valve, *N*, acts as a means by which the pressure within the pneumatic system of the apparatus is maintained or regulated.

*Diastolic Indicator* (According to Fedde).—By means of a plan, embodying a closed metallic reservoir, communicating with a vertical glass tube, containing a freely movable pith ball, we are able to greatly amplify the pulse wave seen in the mercury tube.

This indicator has been arranged to form a component part of this sphygmomanometer. The illustration shows it attached, but it may also be used with other apparatus.

It will be seen by reference to the illustration that the metallic chamber is contained in the base of the case below the working parts of the instrument, and is attached by elastic tubing to the base of the tube communicating with the glass tube of the indicator. The nipple at the base of the indicator serves for attachment of the tube to the cuff, and a short piece of rubber tubing connects the other nipple with nipple *D* of the sphygmomanometer. A careful study of the illustration will show the relation of these parts.

*Determination of the Brachial Blood Pressure—Systolic and Diastolic.*—Place the person to be experimented upon in a comfortable sitting or reclining posture. The instrument should be upon a firm, level surface, within easy reach of both examiner and student. The lid is then raised until it locks in a vertical position, and if the tube from the hand pump, *P*, is not already connected to the nipple, *F*, it should be attached firmly to it. The two

mercury guard cocks, *K* and *L*, on either limb of the U-tube, should be opened and the release valve *N* tightly closed. The hollow rubber tube of the arm-band "*A*" should next be firmly wrapped around the bare arm of the subject above the elbow and securely bound there by the retaining cuff "*B*". This cuff should be applied snugly but without pressure, as it is not designed to compress the member but only to restrain the elastic bag while it is under tension. The tube "*C*" emerging from the arm-band is attached firmly to nipple "*D*," and the stop-cock "*M*" is opened. This arrangement forms a continuous closed pneumatic system, the components of which are the pump or source of air pressure, the compressing arm-band and the manometer tube "*H*."

When pressure is exerted in the arm-band by the air, the amount of compressing force exerted upon the artery, will be indicated by a rise in the left-hand column of the manometer tube "*H*," the height of which will be indicated on the scale "*G*" in Mm. Hg.

*Systolic Reading.*—With one hand find the pulse at the wrist, in the arm to which the cuff is attached. The operator's finger should be in a comfortable position, and under no circumstances should be moved during the observation. It is important to observe that the pulse is not cut off by undue pressure of the palpating finger. With the pulse thus under control, air is pumped into the apparatus until the pressure within the arm-band is greater than the pressure within the blood-vessel, as shown by a failure of the pulse to reach the wrist. When this is accomplished the cock "*M*" is closed. By a fraction of a turn in the valve "*N*," the pressure in the system is very gradually released. During this part of the procedure, a close watch should be kept upon the height of the mercury column for a return of the pulse beat at the wrist. A reading made at the level of the mercury column at this instant will represent the systolic pressure in the vessel of the patient under observation: It is advisable to repeat this procedure several times to insure a correct reading.

*Diastolic Pressure.*—First (Without the Fedde Indicator).

This may be accomplished in two ways. Of these the first will

be found more generally applicable because it does not depend upon the motion imparted to the mercury column by the pulse. After having obtained the systolic pressure and again raised the pressure within the apparatus to the systolic point, keep the fingers upon the pulse, and allow the air to escape from the system very gradually through the valve "*N*." With the return of the pulse it will be noted that at first it is very feeble and thready, and continues so for a time, when suddenly it will assume a full bounding character, somewhat similar to the pulse of aortic regurgitation; at the moment when this change occurs, the level of the mercury column will indicate the diastolic pressure in mm. Hg.

Second Method.—This depends upon the to-and-fro motion imparted to the mercury in the U-tube by the pulse. Having determined this systolic pressure, again raise the pressure to a few millimeters of mercury below this point and immediately close the valve *M*. Now allow the pressure to fall very slowly by releasing the air through the valve *N*, about 2 mm. a pulse beat. It will be noted at this time that the mercury acquires a rhythm synchronous with the pulse. This excursion will gradually increase in amplitude up to a certain point, after which it again decreases and ceases before zero pressure is reached. During this procedure the level of the mercury column at its lower excursion, when it is making its greatest motion, will indicate the diastolic pressure in millimeters of mercury.

*Diastolic Pressure with the Indicator.*—When determining the systolic pressure, pay no attention to this indicator, as each pressure of the pump will make this ball dance up and down, but this has no bearing upon the test. As the systolic pressure is passed, the pith ball should be moving slightly and beating time with the pulse. This motion gradually becomes greater until in some instances the maximum movement amounts to several inches; quite suddenly this excursion becomes markedly less. At the moment of this change the diastotic reading is taken from the height of the mercury column.

The normal cystolic pressure in the radial artery of the adult is about 170 mm. mercury (Potain) at age of 20 to 30 years. It

varies considerably in man with age, increasing from 89 mm. Hg. at age of 10, to 170 mm. at age of 25, then 200 mm. Hg., between age of 40 and 50. The figures of various investigators vary considerably for the same artery, Von Recklinghausen's figure for the radial artery are: systolic pressure 116 mm. Hg. and diastolic pressure 73 mm. Hg. Erlanger gives as systolic pressure in the brachial artery 110 mm. Hg. and as diastolic pressure 65 mm. Hg. at age of 20 to 25 years.

The measurements must always be taken at the level of the heart and with all possible exclusions of psychic influences on part of the subject.

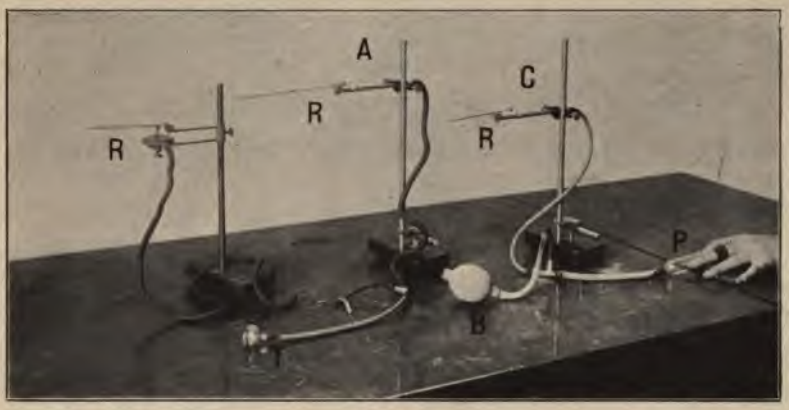



FIG. 38.—*A*, Apparatus to record carotid pressure pulse; *C*, apparatus to record blood pressure in the finger; *R*, recording tambour; *T*, thistle tube; *B*, rubber bulb; *P*, plethysmograph.

*Volume Pulse.*—Place the middle finger in the plethysmograph cylinder, *P*, Fig. 38, having the rubber collar tight enough to retain pressure, but not tight enough to impede the venous circulation. Close the side branch to the tube leading from the cylinder with bull dog forceps. Connect the long tube to the tambour, *R*, Fig. 38, adjust the tambour lever to write on a drum. Periodical alterations, in the volume of the finger, synchronous with the heart beat will be recorded. Note the effect of straining and forced respiration upon the curve. Repeat the experiment with the large plethysmograph inserting the entire forearm

*Blood Pressure in the Finger.*—Squeeze all of the blood out of the middle finger by the application of a one-inch bandage. Apply the rubber collar of the plethysmograph as far as the junction of the first and second phalanges, allowing the bandage to remain around the first phalanx, using the bulbs shown in Fig. 38 at *B;* raise the pressure sufficiently to prevent the return of blood to the finger-tip. Remove the bandage and gradually decrease the pressure until the finger-tip flushes. A mercury manometer is used in this experiment in place of the tambour. The point at which the tip flushes is that at which the pressure of blood in the digital arteries is just sufficient to neutralize the pressure in the sphygmomanometer. The pressure may be read off on the manometer. Record the pressure at the level of the head, heart and knee.

*The Human Pressure Pulse Curve.*—Arrange a drum to revolve slowly (two revolutions a minute); adjust the recording tambour so that its lever will write with the least friction possible, and open the side branch. Adjust a large size thistle tube, *T*, Fig. 37, over the carotid artery, at about the level of the thyroid cartilage, anterior to the sternocleidomastoid muscle. Now with the tambour tight against the artery, an assistant should close the side branch. A sharply marked pulse curve will be recorded. If not the thistle tube is moved into a location that will induce a sharp curve.

Notice the primary wave, the predicrotic elevation, and the dicrotic notch.

By covering the thistle tube with a rubber membrane, having a bone collar button cemented in the center, the pulse in the radial artery may be recorded.

The sphygmograph gives an approximately true record of the form of the pulse, *i.e.*, the time-relations of the changes in blood pressure.

The tambours shown in the arrangement of apparatus in these experiments are delicate. If a stronger one be needed, we make use of the one shown to the left of Fig. 38.

*The Artificial Circulation Scheme.*—The mechanics of the

various parts of the circulatory apparatus of the highest vertebra are illustrated by the artificial circulation scheme.

A pump representing the left ventricle has an elastic outlet tube, similar to the aorta, at the beginning of which is placed a valve

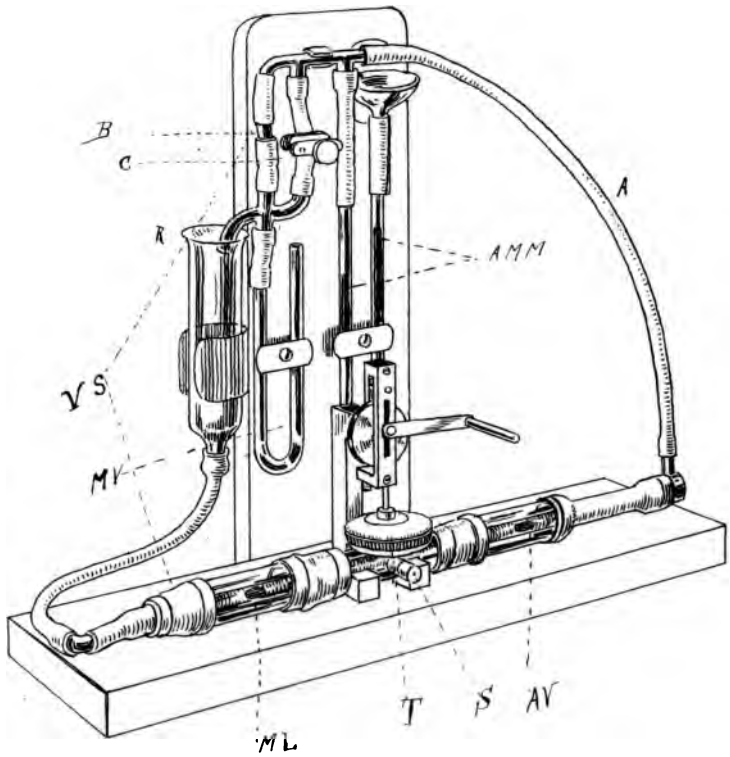



FIG. 39.—Artificial circulation scheme. (*Modified from Porter's Introduction to Physiology.*) *A*, aorta; *B*, bamboo-capillary resistance; *C*. clamp when opened reduces the resistance; *R*, receptable for receiving venous liquid; *VS*, venous system; *MV*, manometer for venous pressure; *ML*, mitral valve; *AV*, aortic valve; *T*, tambour in contact with eccentric; *S*, side opening (kept closed usually); *AMM*, arterial mercury manometer.

representing the aortic valve. The outlet tube leads to two small tubes. One of these tubes has interposed for a small extent of its length a piece of bamboo, the numerous fine channels of which resemble the resistance of the small arteries and capil-

laries. The other tube, or side branch, substituting a wide channel for the narrow ones, is equivalent to a dilatation of the vessels. From either of these two tubes, water flowing in the system, is collected by a single tube, and drops through the air into a receptacle. This water is now returned to the heart by an inlet tube, which has inserted at its junction with the pump a valve, representing the mitral valve.

The pressure in the left ventricle is varied through a tambour, covered with rubber, connected by a rod and disk to an eccentric brass plate which is revolved by hand. Each revolution of the eccentric plate reproduces in the ventricular tube both the time and pressure relations of the ventricular cycle in the animal.

The intraventricular pressure curve is obtained by connecting the side tube to the membrane manometer. A mercury manometer, having a thistle tube at its free end, in order to collect any mercury spilt by a careless operator, records the pressure in the arterial system, near the capillary resistance. A manometer also records the venous pressure near the resistance. During the recording of the intraventricular pressure the arterial mercury manometer should be clamped off.

Normal sphygmographic tracings may be taken from the aorta of this apparatus. The pulse feels similar to the human pulse.

By revolving the eccentric once we can notice a change in pressure in the ventricle, an escape of fluid, a dilatation of the aorta, a change in pressure in the artery, closure of the mitral valve, opening of the aortic valve, and a slight flow of fluid after the revolution.

On increasing the revolutions to about twenty strokes to the minute, the stream becomes intermittent. On further increase in the number of revolutions the flow becomes remittent. Continuing this increase, the flow finally becomes continuous. Notice the changes in blood pressure in these steps, and the fewer number of revolutions required when the bamboo resistance is employed. Notice the limit to which the blood pressure can rise.

The valves should now be observed. The mitral closes as

soon as the ventricle begins to contract, but the aortic does not open until the intraventricular pressure has risen above that in the aorta.

The aortic valve closes when the ventricle begins to relax, but since the intraventricular pressure must fall 100 Mm. of Hg. farther before it shall be lower than that in the auricle, the mitral valve does not open. During this fall the heart valves are again closed.

Through the ventricular manometer and a sphygmograph, the aortic pulse and the intraventricular pressure may be recorded by this scheme.

By removing the rubber from about the aortic valve we can bring about a condition in which the valve is insufficient. This condition is called aortic regurgitation and is caused in man by disease. Notice the manometer, record pulse curves and feel the character of the pulse beat.

Replace the rubber flap and tie a string around the flap and tube just over the opening in the tube. Stenosis, *i.e.*, narrowing of the tube is produced. Disease usually causes this condition in man. Observe conditions as before.

Perform the same operations on the mitral valve and note the results. (Description modified from Porter, l. c.)

## Investigation of the Blood Pressure in the Carotid of the Rabbit.

*Apparatus.*—Ten per cent. chloral hydrate solution, graduated cylinder, NaCl solution, scale, soft catheter, olive oil, small funnel, manometer, revolving drum, carotid cannula, rubber tubing, half-saturated solution of $Na_2CO_3$, normal saline, dissecting instruments, as knife, forceps, tenacula, scissors, retractors, blunt hooks, aneurysm needles, artery forceps, small needles, needle holders, small forceps, rabbit board, 2 per cent. potassium oxalate solution, pipette, inductorium, cells, key, tuning fork, clamp, stand, needle electrodes, syringe. Hirudin 1/10 gram.

*Large Mercury Manometer.*—A glass U-tube mounted upon a board to which is screwed a rod to be clamped in a stand (Fig. 39).

The hard rubber float is hollowed to fit the meniscus. The other moving parts are aluminium. A brass stop-cock greatly facilitates filling and cleaning the manometer, as well as the making of pressures preliminary to opening the connection with the artery.

*Narcosis of the Animal.*—Measure off from a 10 per cent. watery chloral hydrate solution in a small graduated cylinder a quantity in the proportion of 3 cm. of the solution to 1 kilo body weight of the animal. Raise the animal by its hind legs, introduce into the rectum a soft catheter, the point of which is first dipped in olive oil, and pour the chloral hydrate solution into a small funnel which is inserted in the outer end of the catheter. After the solution has run into the intestine, slowly draw out the catheter; after one-fourth of an hour narcosis will be noticed. In the meantime prepare the manometer and the recording apparatus.

A revolving cylinder is covered with smoked paper; a mercury manometer with an upright and writing style resting on the mercury of one glass limb of the U-tube and adjusted to record on the kymograph; the other end of the manometer is connected through pressure tubing with the carotid cannula.

The proximal limb of the manometer, and the tubing connected with it, are filled with a half-saturated solution of sodium carbonate, a magnesium sulphate or some other salt solution to prevent clotting of the blood which may find its way into the tubing. Hirudin or extract made from the pharynx of the leech is very effective to prevent clotting.[1]

The pressure in the manometer and connections is raised to approximate the arterial pressure of the rabbit. To prevent forcing the metallic mercury out of manometer this must be clamped off.

Draw a base line around the drum to indicate the atmospheric pressure before the mercury in the manometer is put under pressure. The height of the tracing above this line, multiplied by 2, gives the pressure in terms of mm. of mercury.

[1] 0.1 gram of Hirudin is dissolved in 25 c.c. water. Ten c.c. of this solution will suffice for an ordinary rabbit.

Arrange the metronome to record seconds and the drum should revolve at slow speed. Place in connection in the primary circuit apparatus necessary for the production of rapidly succeeding induction currents, and also the simple key. The needle electrodes are arranged as in the previous experiments.

**Strapping of the Animal.**—Place the animal belly down on the rabbit board, tie the legs with cords, which are fastened to the screw on the side of the rabbit board. Support the head on a wire shaped like a horseshoe, which holds the neck behind the ears. From the head supporter a rod with a movable ring projects across the forehead of the animal; the latter is pushed over the mouth and fastened by a screw to the rod. The latter is attached in front to an upright stand on the rabbit board. Fasten the animal in such a way that the neck is freely exposed for the operation. Remove the hairs on the neck with scissors.

**Operation.**—Make a long incision in the median line of the neck from the thyroid to the breastbone. The edges are kept separated by assistants with retractors or blunt hooks. The incision is carried far through the skin muscle and the superficial fascia, and hemorrhages from larger vessels are controlled by clamp forceps and ligatures.

Determine now the position of the sternomastoid and sternohyoid muscles (*s.m.* and *s.h.*, Fig. 40).

With a blunt instrument (scalpel handle or seeker) carry the incision into the groove at the inner edge of the sternomastoid and expose the attachments in this groove. The carotid artery is at once detected. This is laid free with blunt instruments as far as possible. Draw two threads under the free prepared portion of this artery. Bind together with the one thread the cephalic end of the exposed part; the other thread serves later for the tying of the cannula.

## Method of Inserting a Cannula into Carotid Artery.

Clamp the prepared artery toward the heart with a small forceps. Place a waxed ligature around the vessel distally from this clamp. Now grasp the carotid about the middle of the free portion with

a pair of forceps and cut with the scissors a small hole in the wall of the vessel above the portion grasped with the forceps. In the tying in of the cannula into the carotid it is best to cut the opening into the artery in the shape of a triangular little flap or ear on the upper side of the vessel—an assistant holds the sides of

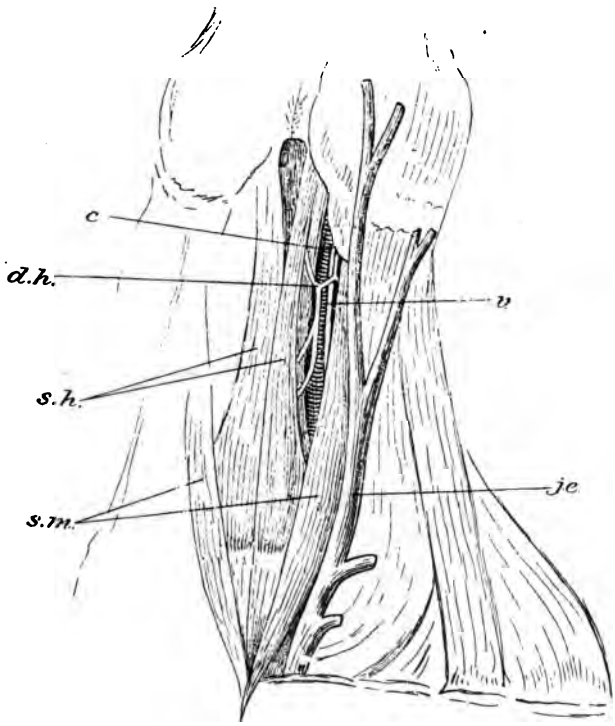



FIG. 40.—Topography of rabbit's neck: *s.m*, sternomastoid; *s.h*, sternohyoid; *c*, carotid artery; *j.e*, jugular vein; *v*, vagus nerve; *d.h*, ramus descendens hypoglossi.

this cut apart with two fine forceps, whilst the operator inserts the cannula in the direction toward the heart. The *cannula* is a small tube of glass or metal; that end which is to be inserted into the artery is drawn out to a fine point and the other end is connected with the glass tube of the manometer by rubber tubing. The cannula before it is inserted into the artery must be filled with

a 2 per cent. potassium oxalate or hirudin solution (delaying coagulation). Tie the wall of the artery to the cannula with the second thread. In order to prevent slipping of the thread, the drawn-out end of the cannula is provided with a groove, into which the thread is securely bound. By means of a small pipette replenish the cannula with oxalate solution, if there should have

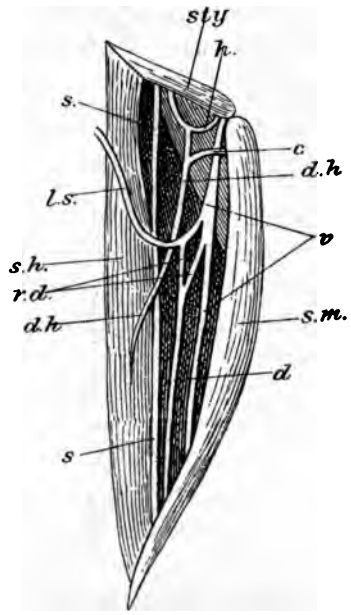



FIG. 41 —Scheme of the cervical nerves of the rabbit: *v*, vagus; *s.s*, sympathetic; *h*, hypoglossal; *d.h*, descendens hypoglossi; *d*, depressor; *r.d*, root of depressor; *l.s*, superior laryngeal; *c*, connection between hypoglossal nerve and brachial plexus; *s.m*, sternomastoid; *s.h*, sternohyoid; *sty*, stylohyoid.

been some loss at the insertion of the cannula into the artery. Now expose also the vagus nerve to some extent. This nerve lies to the side of and immediately behind the carotid (*v*, Fig. 32). It is the thickest among the nerves there. Pass a fine thread through under the vagus (compare also Fig. 41).

**Connection of the Cannula with the Manometer.**—Place the rabbit board so high that the cannula is on a level with the

rubber membrane of the manometer. Apply the recording manometer to the smoked surface. Revolve the drum once before the rubber hose of the cannula is pushed over the glass tube of the manometer. The recorder marks then a horizontal straight line which serves as a base line for the blood-pressure curve. Now apply the rubber tube of the cannula over the glass tube of the manometer, but in such a way that no air bubble is enclosed. Remove the small forceps from the artery. The blood pressure exerts its effect upon the manometer.

## Recording the Blood Pressure Curve. Influence of the Vagus on the Heart.

Let the writing drum revolve somewhat and record a normal blood-pressure curve. Then stop the drum. Tie a thread under the vagus as high as possible over the nerve and cut the nerve above (cephalad) this point. Place the peripheral stump of the vagus upon the electrodes and stimulate by opening of the circuit while the drum is revolving. In this manner make several simultations with the coils each time at a greater distance from each other, and register the effect of each stimulation. Should the effect not be clearly noticeable, prepare the other vagus and stimulate it.

After completion of the stimulating experiments apply again the small forceps to the artery, remove the rubber hose of the cannula from the manometer tubing and let the animal bleed to death from the cut carotid. Keep the manometer and the writing drum undisturbed for the gauging. For this purpose connect the free end of the glass tube of the manometer through rubber tubing with the manometer employed for the estimation of the human blood pressure. The latter is connected with the syringe used for the increase of pressure. By means of the syringe increase the pressure in these parts. The increased pressure is indicated on the mercury manometer in millimeters of mercury. The *rubber* manometer indicates through its position of its hand the corresponding ordinate height, which is recorded by letting the drum revolve somewhat. Make several such gauging experiments at various

pressure values, determine from the obtained figures the proportional pressure, measured by a mercury column in millimeters to 1 mm. ordinate height and take the obtained number into account in the estimation of the various parts of the blood-pressure curve.

Repeat all the experiments on the rabbit's vagus that have been described in detail and executed on the frog.

# CHAPTER VIII.

## RESPIRATION.

### Spirometer. Experiments Concerning the Cervical Sympathetic and the Breathing Innervation of the Rabbit.

*Apparatus.*—Spirometer, 10 per cent. chloral hydrate solution, NaCl solution, soft catheter, olive oil, small funnel, dissecting instruments, as knife, scissors, forceps, tenacula, retractors, blunt hooks, ligatures, aneurysm needles, artery forceps, small needles, needle holders, small forceps, rabbit board, 2 per cent. potassium oxalate solution, pipette, inductorium, needle electrodes, dry cell, simple key, syringe, glass tracheal cannula, tubing, T-tube, small flask of 500 cm. capacity, double perforated stopper, glass tubing, manometer, recording apparatus, bellows, mouth-piece.

The spirometer serves for the determination of the respiratory capacity. It consists of an outer cylindrical vessel, filled with water, and an inner cylindrical vessel which is balanced by weights, hid in the uprights at the side of figure 42.

Air is expired into the mouth-piece shown at the front of the figure, and enters the second cylinder through the rubber and metal tubing. As the air displaces the water in the inner cylinder, the cylinder rises. A pointer placed at the top of the inner vessel indicates on the upright to the left of the figure the number of cubic centimeters of the air expired or inspired.

Before the beginning of the experiment the inner cylinder must stand at the zero mark of the scale. In order to remove the air after an experiment, pull a plug from a hole in the lid of the inner vessel, push the cylinder down to the zero mark, and insert the plug again into the hole.

**Tidal Air.**—Expire into the spirometer clylinder after an ordi-

nary inspiratory effort. This record of the expired air is an approximate indication of the *tidal air*, *i.e.*, the amount that passes in and out of the lungs during quiet respiration (400 to 500 c.c.).

**Supplemental Air.**—Take a normal inspiration and then force out as much air of the lungs as possible. Record the amount on the spirometer scale. Subtract the reading of the former experiment from the last reading. The difference is the *supplemental* or *reserve air* (1500 c.c.).

The air which can be inspired in addition to the ordinary inspiration is known as the *complemental air* (2000 c.c.).

**Vital Capacity.**—Inspire as deeply as possible and breathe into the spirometer as completely as possible (3500 to 4000 c.c.).

The *residual air* is the amount of air that remains in the lungs after the most violent expiratory effort (1000 to 1200 c.c.).

The *vital capacity* represents the full capacity of the lungs minus the residual air, and equals the sum of the tidal air, complemental air and supplemental air.

FIG. 42.—Spirometer.

## Section and Stimulation of the Cervical Sympathetic of a Rabbit.

Strap a rabbit which is under the influence of chloral hydrate back down upon the rabbit board; perform the operation as in the

sixteenth lesson, but do not injure the carotid. Locate behind the carotid and the vagus the cervical sympathetic nerve; between it and the vagus lies also the depressor, which is to be left intact.

Now secure the cervical sympathetic nerve on one side with two ligatures and cut the nerve between the ligatures.

Hold both ears of the animal so that the daylight passes through them and note that the vascularity of the ear on the side where the nerve was cut is greater, also that the temperature of the ear on the operated side is higher than that of the ear on the sound side.

## Vaso-dilator Effect of Cutting Cervical Sympathetic in Rabbit.

Now draw the upper stump of the cut sympathetic on the thread upward and place it on two needle electrodes. Arrange an inductorium for stimulation as in the stimulation of the vagus in the previous experiment (p. 114). Stimulate the nerve and first observe the ears during the stimulation. Note the diminished vascularity on the stimulated side.

Stimulate again and observe the pupil on the stimulated side. Does it become larger or smaller?

## Experiments on the Breathing-innervation on a Rabbit. Graphic Recording of Respiration and of Experiments on the Respiratory Organs.

**Arrangement of Operation.**—Expose the trachea in the same rabbit that was used in the previous experiment by a longitudinal cut through the muscles that lie above it and separate it with a blunt instrument from its attachments. Pass a needle under the trachea, thread the needle, and pull the thread through under the trachea by withdrawing the needle. Cut the wall of the trachea half through with a knife in front of the thread. Insert in the incision a suitable glass cannula and tie the wall of the trachea firmly with the thread on the neck of the cannula. Connect the outer end of the cannula by short tubing with the first limb of a

T-tube. Place at the side of the rabbit-board a glass flask of 500 c.c. capacity, in the neck of which is placed a doubly perforated stopper; in each hole of the stopper a small glass tube is inserted; connect the one glass tube by rubber tubing with the second limb of the T-tube on the tracheal cannula; the second glass tube is connected with rubber tubing to the Marey Tambour (Fig. 29). Arrange the tambour to write on the kymograph.

The flask interposed between the tracheal cannula and the recording drum serves the purpose to delay the decrease in O and overcharging with $CO_2$ in the tubing during the recording of the respiratory movements. Arrange the metronome to record seconds.

**Procedure.** 1. *Registration of the Normal Breathing, Eupnea.*—Apply to the third limb of the T-tube at the tracheal cannula a short piece of rubber tubing, which is closed by a strong clamp; immediately afterward set the clockwork in motion.

Determine by the recorded curve the rate of the normal breathing. After the registration of some respirations remove the piece of rubber hose with the clamp again and stop the clockwork of the kymograph.

2. *Registration of the Breathing Pause after Strong Artificial Respiration, Apnea.*—Attach now to the free limb of the T-tube at the tracheal cannula a somewhat longer tube, which has an opening in its side wall not far from the T-tube and the other end of which is connected with a small bellows. With this blow air into the lungs of the animal in a somewhat quicker rhythm than the corresponding respiratory movements of the animal; the lungs become inflated, but the air can always escape again through the hole in the rubber hose. Continue with the artificial respiration for about one minute, then immediately close the rubber tube by applying the clamp between the T-tube and hole and set the clockwork at once in motion. The breathing of the animal ceases then for some time and gradually begins again, first with feeble respirations, which gradually increase in strength more and more. Then stop the clockwork and remove the rubber hose from the T-tube.

3. *Hering-Breuer's Method for Inflating the Lungs.*—Let the animal breathe freely again in the open for some time; apply the rubber tube with the hole once more to the T-tube; its free end, however, is not connected any more with the bellows, but is provided with a mouth-piece. The clockwork is started and the operator blows through the mouth-piece moderately strong into the lungs of the animal. Apply the clamp forceps to the rubber tube between the T-tube and the hole when the lungs are inflated. During the inflation the recorder has risen and remains high. Note as an effect of the inflation that the breathing ceases for some time. Later on, when the movements of the recorder indicate the return of breathing, remove again the clamp forceps and the rubber tube with the hole.

4. *Section of Vagus.*—Now expose and isolate on both sides the vagi of the animal and cut through with a pair of sharp scissors. Register the respiration as in Experiment 1. Note that the respirations have become less frequent, but deeper. The cessation of breathing cannot be effected any more through inflation of the lungs in the manner described after the vagi are cut. The experiment being completed, the apparatus is taken apart; the animal is bled to death by incision of the carotid and the smoked paper is varnished.

The respiratory movements may also be recorded in animals by inserting a rubber bag between the pleural surfaces. Also by introducing a catheter into the right or left bronchus. The bag has attached to it a rubber tube which is connected with a tambour and a pump to expand the rubber bag through a cannula.

The pneumograph is a tambour-like arrangement which can be strapped around the human chest to record respiration.

The chest pantograph devised by Prof. Winfield S. Hall should be demonstrated to the class by the instructor and the external thoracic contours of 2–3 students graphically recorded by this apparatus.

# CHAPTER IX.

## NERVOUS SYSTEM.

### Determination of the Reaction Time. Experiments on the Brain and Spinal Cord of the Frog.

*Apparatus.*—Two short circuiting keys, signal magnet, dry cells, inductorium, drum, tuning fork, clamp stands, cotton, dissecting instruments, acetic acid, forceps, needle electrodes, 1 per cent. solution of strychnine.

A. **Determination of the Reaction Time.**—The subject after stimulation of the sensory nerves of a hand shall execute as quickly as possible a movement with the other hand. Register the moment of stimulation and the beginning of the movement of the other hand and estimate in this way the time which passes from the stimulation until the beginning of the movement, or response.

*Arrangement of the Experiment.*—Two short-circuiting keys are placed in circuit with the signal magnet so that by closing either one, the signal magnet may be cut out of the circuit. The subject of the experiment grasps the handle of one of these keys in his right hand and places two fingers of the left hand slightly moistened on the polar screws of an inductorium, which is included in the circuit of the other key. The inductorium is arranged to give single induction shocks. Beneath the tracing on the drum is arranged a tuning fork to record the time in one-hundredths of a second. In order that he may not be disturbed by other observations, the subject closes his eyes and puts cotton in his ears and concentrates his attention on the stimulation of the finger nerves.

*Demonstration.*—Use a rapidly revolving drum. Close the primary circuit. At the moment of stimulation of his finger, the subject should close his short-circuiting key. By this arrangement

the moment of stimulation is recorded on the drum and the moment of response, as indicated by the closing of the second circuit, is also recorded. Calculate the time between the two. The interval between the opening of the circuit and its closing represents the time occupied for the sensory impulse to pass to the sensory centers in the brain; its transfer to a motor neuron; its passage to the muscles involved, and the latent period of these muscles. What is a reflex? Describe varieties of reflexes.

B. **Experiments on the Frog's Brain and Spinal Cord.**—Figs. 43 and 44 explain the anatomy of the skull and brain. The brain

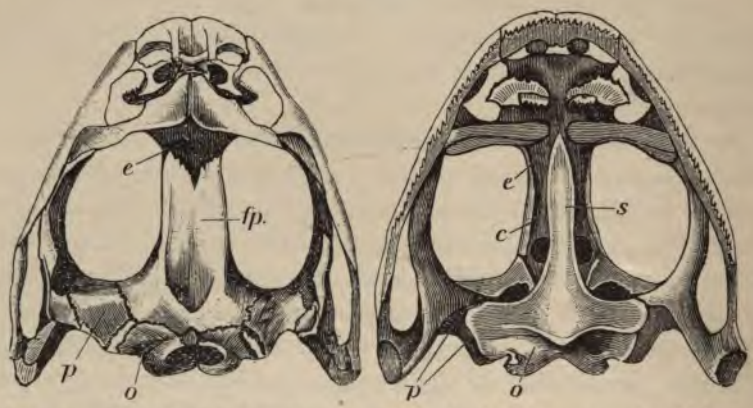



FIG. 43.—Anterior and posterior views of the frog's cranium; *e*, ethmoid bone; *fp*, frontal and parietal bones; *p*, petrous bone; *s*, sphenoidal bone; *o*, occipital bone; *c*, cartilaginous side wall of the skull.

consists of three sections: 1. the fore-brain, with the hemispheres *H* and *H*, and with the intermediate brain (thalamus); 2. the mid-brain or optic lobes *L* and *L*; 3. hind-brain with the process corresponding to the cerebellum. In order to facilitate the learning of the topography of these parts, it may be mentioned that a line which connects the anterior edges of both tympanic membranes (visible outside on the frog's head) strikes a little in front of the border between the fore- and mid-brain. A line connecting the posterior edges of the tympanic membranes strikes somewhat behind the border between hind- and mid-brain.

*Separation of the Fore-brain.*—Cut transversely through a frog's head with scissors in a line which lies behind the conjunction of the anterior edges of the tympanic membranes. The frog minus the fore-brain, *i.e.*, the organ of sensation and government of voluntary movements, acts yet as a normal frog, but he does not perform spontaneous movements any more. If he is placed on his back, he straightens up; if he is touched, he jumps away.

*Removal of the Entire Brain.*—Now sever the entire head close in front of the shouler blades. The frog now has lost the reaction

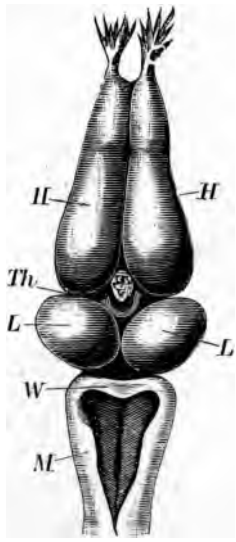



FIG. 44.

of the normal animal, he does not straighten himself when placed on his back. However, upon stimulation of sensory nerves, the decerebrized frog still executes a series of attempts to brush off offending irritants. When the foot, for example, is squeezed by forceps, he tries to push away the instrument. When a part of the skin is touched with filter paper dipped in dilute acetic acid, he wipes off that part. Execute these experiments.

*Stimulation for Reflex Action.*—Arrange an inductorium for stimulation with single induced currents. Attach needle

electrodes to the secondary coil and apply them to a part of the frog's foot. Even with a strong current the reflex actions cannot be obtained any longer. Now arrange the inductorium for frequent stimulation and apply the electrodes. Even with a weak current reflex movements will be obtained.

*Poisoning with Strychnine.*—Inject a few drops of a 0.1 per cent. solution of strychnine (precaution, strong poison) under the dorsal skin of a frog (as in the poisoning with curare). After a few minutes the action of the poison is apparent. Upon the slightest stimulation (the mere touch of the skin) the frog is thrown into tetanic spasms and convulsions. Upon which part of the nervous system has the strychnin acted.

# CHAPTER X.

## VISION.

### Ophthalmometer, Purkinje-Sanson's Images, Visual Purple.

*Apparatus.*—Ophthalmometer, phacoscope, dissecting instruments.

### Ophthalmometry.

Ophthalmometry, as its name indicates, is that branch of science which is concerned with the measurement of the various refracting media and structures of the eye. This book only takes up a description of the method of ascertaining the measurements of the curvature of the anterior surface of the cornea in its different meridians.

The ophthalmometer is the name given to the instrument used in this science. Helmholtz invented the first ophthalmometer, but it was superseded by the instrument of Javal and Schiotz. Now the instrument in favor in the United States is the C. I. ophthalmometer.

A small inverting telescope, mounted on a rod which can be moved up and down inside a cylinder-like arrangement, constitutes an essential part of the ophthalmometer. The compound objective of the telescope consists of two convex lenses between which is placed a doubly refracting prism.

The eye, the cornea of which is to be measured, is placed at the the principal focus of the lens *nearest the eye of the observed one.* At the principal focus of the other lens, there is formed an inverted image of the same size as the erect image formed by reflection on the cornea. The purpose of the doubly refracting prism is to increase in size the image formed on the cornea.

The image used is shown in Fig. 45, one being placed above and one below the telescope on the concave surface of the disk. These sights (mires) are stationary, and are in the same plane as the deviation of the bi-refringent planes. They are translucent and

FIG. 45.—Ophthalmometer.

are illuminated from behind by incandescent lamps, contained within cups on the back of the disk. We effect the position of the reflected images of the mires as seen by the operator on the cornea by the position of the prisms, which are fitted in an inner tube,

and carried back and forth at will by means of a finely adjusted rack and pinion. The effect of moving the prisms longitudinally is to approximate or separate the images of the mires as seen on the cornea.

To the pinion are attached graduated wheels or indicators giving the radii in tenths of a millimeter and the equivalent value in the telescope at the eye. Raise or lower the instrument until the top of the patient's cornea may be seen over the end of the tube, or until the eye and the telescope are on a level.

*Focusing the Telescope.*—Get the patient to open his eyes widely and to look steadily into the tube. Look through the telescope at the uncovered eye, and focus it until a clear, sharp image of the mires is seen. Readjust the instrument again if necessary. An outer image may be seen on either side of the field of view, but these are always widely separated from the inner ones, and are to be disregarded.

*Locating the Principal Meridians.*—Rotate the telescope to the right or left until the mires lie in a meridian where the two long meridian lines show a single, straight and unbroken line. If there is no astigmatism this condition will be seen at all axial positions; if regular astigmatism is present, at but two positions.

When these lines form one unbroken line, one meridian has been located. This is called the primary position.

*Measuring the Astigmatism.*—After a meridian has been located, revolve the graduated wheel at the sides of the telescope until the short lines, or spurs, of the images unite and form a perfect cross. Now adjust the movable index pointer attached to the left wheel so that it is coincident with the stationary one below the telescope. It then records in diopters and fractions of a diopter the regular refracting power in the primary position.

Rotate the telescope 90 degrees, or to the secondary position. (The perforated meridian pointer indicates the meridian for secondary position.) If there is any corneal astigmatism present, the spurs will have moved away from each other.

They should now be adjusted as before until a perfect cross has been obtained.

The stationary pointer now indicates the refraction in the secondary position, and the movable pointer indicates the refraction in the primary position. The number of whole diopters and fractions of a diopter between these two pointers is the amount of the corneal astigmatism, and if irregular astigmatism is present there will be a broken line in all axial positions.

To determine whether an eye is myopic or hypermetropic we make use of figures which indicate that the average curvature is represented as 45D. On this basis, the theory has been advanced that a cornea showing a curve greater than 45D. will indicate myopia, while a cornea of lower curve than 45D. will indicate hypermetropia.

B. **Purkinje-Sanson's Images.**—These are the images formed by the cornea, the anterior curved surface of the lens and the

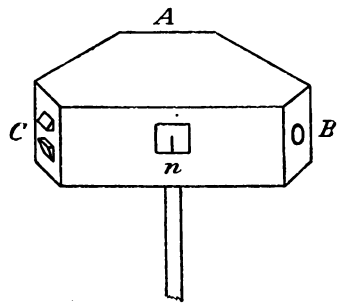



FIG. 46.

posterior surface of the lens. They are observed by Helmholtz's *phacoscope*. This consists of a case formed like a flat triangular prism, Fig. 46, with three obtuse edges. At the latter there are holes, one for the eye to be observed (on the side at *A*, Fig. 46), one for the observing eye (at *B*), and one for the light emitting object (at *C*). In the hole at *C* are inserted two separated prisms in such a way that if both prisms are illuminated by a flame, they appear as two equally illuminated squares, seen from *A*. In the side wall of the case, opposite to the eye to be observed, there is a fourth hole in which a needle is attached. Fig. 47 explains the

experimental arrangement still better. In the dark room the case is placed on a table and at *C* (close to the prisms) a lighted candle is adjusted; the subject applies his eye at *A* and gazes along needle *n* at a far distant point *f*. At *B* is the eye of the observer, who looks now into the eye of the subject and sees the three images depicted in

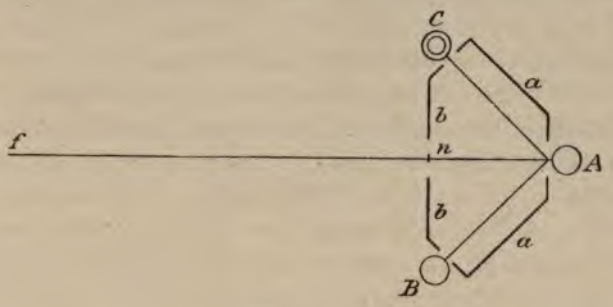



FIG. 47.

Fig. 48, *A* (at *a* the small, erect image formed by the anterior convex surface of the cornea, at *b* the larger and less distinct image, formed by the anterior convex surface of the lens, at *c* the smaller inverted and indistinct image formed by the posterior


FIG. 48.

surface of the lens). Now let the subject fix his vision upon the needle *n*. Thereby the images are changed as in Fig. 48, *B*.

C. **The Visual Purple.**—With a frog which has been kept for twenty-four hours in the dark make the preparation in red light. With scissors bisect the head in two transverse sections, the first

time close in front of the eyes, the second time close behind the eyes. Grasp the isolated middle part of the head between the thumb and index-finger in such a way that an eye is pressed out between the fingers. Bisect this eye equatorially and detach the retina from the posterior part of the eye cavity by means of a pair of forceps. Place the retina on a porcelain plate and examine it by daylight. The retina is found to be of a red color; but after a short exposure to daylight it fades rapidly.

All measurements and studies of pathologic conditions and phenomena of the eye should be executed in the clinic and dark rooms of the ophthalmologists.

# CHAPTER XI.

## FERMENTATION, FERMENTS, ENZYMES AND CATALYZERS.

Fermentation must not be regarded merely as a decompostion and transformation of fermentable substances outside of the body by bacteria or fungi—nor even as limited to the transformation of food substances in the digestive tract—but in its broader biologic sense fermentation is a phase of cellular nutrition. The term has been extended to include all of those phases of the nutrition of unicellular and multicellular organisms which involve the consumption of complex substances and the excretion of simpler ones.

Summing up his studies on the subject of yeast fermentation Pasteur said (Comp. Rend. de l'acad. des Sci., Vol. LXXV, p. 784): "The weight of yeast which is produced under these conditions, that is, in the presence of free-oxygen gas, during the decomposition of sugar increases progressively, and approaches the weight of the decomposed sugar in exact proportion as its life goes on in the presence of increasing quantities of free oxygen. Guided by these facts I have been gradually led to look upon fermentation as a necessary consequence of the manifestation of life when that life goes on without the direct combustion due to free oxygen. We may see as a consequence of this theory that every organism, every cell which lives or continues its life without making direct use of atmospheric air, or which uses it in quantities insufficient for the whole of the phenomena of its own nutrition, must possess the characteristics of a ferment with regard to the substances which are the source of its total or complemental heat.

### Catalysis.

**Catalyzers.**—In 1835 Berzelius expressed his conception of catalyzers as bodies which can awaken the slumbering

chemic affinities of substances by their mere presence. Ostwald, in an essay on "Catalysis" (1901), defined a ferment or catalyzer as a substance which can cause a chemical transformation merely by its presence without itself participating in the reaction, which would take place without the ferment, but only very slowly.

According to Ostwald a ferment only influences the time in which a chemical reaction occurs. It either accelerates it, or in rare cases slows it, without appearing in the end-product of the reaction. A catalyzer or an enzyme does not effect chemical transformation which could not also take place without these agents, by other means, but it only changes the time in which these reactions occur, according to Ostwald.

These are not definitions attempting an explanation of enzyme action, but simply giving a description. The term "catalyzer" is generally applied to the inorganic agents of this class, as a type of which we might quote platinum black or colloidal platinum. Other inorganic ferments or catalyzers are the ions of hydrogen and palladium.

If we conceive of catalysis in the sense of Ostwald, as a change in the rate of a chemical reaction, by a substance which does not appear in the end-product of that reaction, then catalysis is a very widely distributed phenomenon and according to Ostwald there is no kind of a chemical reaction which could not be influenced catalytically, and there are no kinds of chemic substances, whether they are elements or compounds, which could not under certain conditions act catalytically.

Bredig and his pupils have measured the manifold and energetic catalytic effects which can be exerted by colloidal platinum and other colloidal metals, and he has repeatedly emphasized that the physiologic catalyzers, the enzymes, are always in a condition of colloidal solution or suspension. (Ergebnisse d. Physiol., B. I, 1902, p. 134.)

The term colloid (proposed by Thomas Graham, 1861), designates substances that are practically incapable of diffusion through porous membranes. Substances like salt, sugar, acids, etc.,

which diffuse readily, are called crystalloids. Colloidal substances are regarded by Quinke as fluids in which there are numerous invisible thin partitions of firm or fluid lamellæ—the structure being likened by him to that of a sponge filled with water.

A further characteristic property more or less common to all colloids is the peculiar transformation they can undergo from a colloidal dissolved form to a gelatinized form—a phenomenon which is designated as coagulation or gelatinization. In the colloid dissolved form they are spoken of as "Sol"; in the gelatinized form as "Gel," ex. Platin *Sol* and Platin *Gel.*

For example gastric juice is a *sol* of the organic colloids—pepsin and chymosin in 0.2 per cent. of HCl.

Organic colloids embrace all albuminous substances of animal and vegetable origin, the native albumins as well as the albumoses and peptones; further, all glue-like and gelatinous substances, the higher carbohydrates like starch, rubber, dextrine, etc., all enzymes, perhaps the soaps and all pigments of organic origin.

The physiologic catalyzers, or the enzymes, are bodies which arise in the organism during the life of the cells, and by the effects of which living things transact the greatest part of their chemical work, not only digestion and assimilation are governed from beginning to end by enzymes, but all the fundamental intracellular life activities of organisms are transacted under the decisive coaction of enzymes, and would be impossible without these.

I refer more especially to the elaboration of the requisite chemical energy through oxidation, at the expense of the oxygen of the air: For the free oxygen is, as is well known, a very indolent and slow agent at the temperature of the organisms and without an acceleration of the rate of its reaction by some catalyzer, the maintenance of life would be impossible. In this way hemoglobin and hematin act as catalyzers.

If we should ask the fundamental question, "What are the physico-chemical exponents of life phenomena?" the answer

would be "The physico-chemical characteristics of life phenomena are automatic and self-regulating acquirement, application and utilization of chemical and physical energy for the activity, maintenance and multiplication of the living organisms."

Now there are only three means by which living organisms could initiate or influence the celerity of chemical reactions: 1. By temperature, 2. by concentration and 3. by catalyzers or enzymes. Of these three, the first, viz., temperature, cannot be held or maintained at any desirable degree, or sufficiently high degree in the living organism sufficient to start or accelerate chemic reaction, for warm-blooded living organisms possess extremely delicate mechanisms by which their temperature is kept automatically within rather narrow limits.

These thermostatic provisions always keep the body temperature within very narrow limits during life. The second means, or the concentration of solutions, is limited by the solubility of most substances, and also by the fact that concentration of reagents beyond certain narrow limits is destructive to protoplasm. Therefore, the only universally applicable means to regulate the onset and rate of chemical reaction are the enzymes or catalyzers.

To designate the organic ferments as catalyzers is, however, not a justified nomenclature if the term is used to mean simply an accelerator. For the organic enzymes are more than accelerators—they actually initiate the process that is peculiar to their specific work. Solutions of sugar, albumins and peptones do not undergo splitting up or oxidation spontaneously and have been kept in an undecomposed state for years; but on contact with the proper enzyme they underwent hydrolytic cleavage at once.

Recent work by Abderhalden and Gigon (Zeitschr. f. physiol. Chemie, 53, 251, 1907) also indicates that ferments actually enter into a chemical union with the *substrate* but this union is dissolved again and this is the reason why they were believed to act catalytically, and that they could transform an enormous quantity of the substrate compared to their own weight.

The quantitative determination of ferments is exceedingly difficult because they have not yet been isolated in the pure state

and their presence can only be ascertained by their specific actions.

It was formerly believed that synthetic effects could not be produced by enzymes, that they could only break down and decompose and could not build up. This has been disproved by the fundamental experiments of Kastle and Loevenhart (*Amer. Chemical Journal*, Vol. XXIV, p. 491, 1900) who demonstrated conclusively that lipase, a fat-splitting ferment, could not only decompose ethyl butyrate into ethyl alcohol and butyric acid, but reversely could form ethyl butyrate from these products. This is spoken of as the "Reversibility of Enzymes" and it is a possible factor in the digestion and absorption of fats in the human intestine. For if steapsin, the lipase of pancreatic juice, cannot only split up neutral fats into fatty acids and glycerine, but can also recombine fatty acids and glycerine and reform neutral fats, the absorption of fat in the human intestine has received a simple explanation. For in the chyle of the thoracic duct we find simply fats in very fine suspension, and no fatty acid and glycerine, and it is possible that the same ferment which has originally split up the neutral fat has after absorption, recombined the glycerine and fatty acids into neutral fat again.

Croft Hill was really the first one to demonstrate the reversibility of enzyme reactions (*Journal of the Chemical Society*, No. 73, p. 694, 1898), and Ewald in 1883 showed (*Archiv. für Physiologie*, Sup. 302) that when one adds dried intestinal mucosa to fatty acid and glycerine, fat will be formed.

The action of enzymes is arrested by their own products, but when such an arrest of ferment action has taken place, it can be made to resume its work 1. by adding more of the reacting substances, meaning the enzyme; 2. by removing the products of the reaction; 3. by diluting or concentrating the solution; 4. by changing the temperature.

The student should prepare himself by reading up the following subjects:

**"Anti-catalyzers or Enzyme Poisons and the Specificity of Enzyme Reaction."** By G. Bredig (*Ergebnisse d. Physi-*

*ologie*, Jahrgang 1, Bd. I, S. 134–209); also on the "Significance of the Intracellular Ferments," by Martin Jacoby (*Ergebnisse d. Physiologie*, B. I, S. 213–242).

Enzyme poisons; Anti-enzymes; Anti-ferments secreted by intestinal parasites; Application of Ehrlich's side chain theory to ferment action (Starling, "Recent Advances in Physiology of Digestion," p. 35); Increase of surface by reducing a metal to a colloidal state (Starling, p. 15, *l. c.*).

Among the most interesting facts of these studies are the evidences that there are substances that can render enzymes active, if they are formed in an inactive state (trypsinogen) and substances that can render them inactive, and furthermore, that there are enzyme poisons and catalyzer poisons. Example, HCN. Make a solution of 0.0015 mg. of HCN in one liter of water, observe the inhibition or arrest of the catalytic effect of colloidal platinum on $H_2O_2$. But the organic enzymes are also poisoned by HCN just like colloidal platinum is. Both may, however, gradually recover from the poisoning if the HCN is removed.

$H_2S$, $CS_2$ and CO also have similar poisonous properties on enzymes.

## Characteristics of the Ferments of Digestion.

Ferments can filter through clay and porcelain but are not dialyzable; they are destroyed by heating up to 60° to 80° C. when moist but can stand a higher temperature when dry. A characteristic is that they become attached to all solid precipitates. Pure ferments give no characteristic reaction; the precipitates produced by iron, tannic acid, etc., are caused by impurities. Ferments stand in close stereo-chemical relation to the structure of their substrate. Ferment and substrate stand in a similar relation to each other as a key to the lock (Emil Fischer). The amylolytic enzymes fit only to one optic antipod of two kinds of sugars —either to dextrose or levulose—not to both. The same is true of the proteolytic enzymes. Ferments that act upon optically active material (*i.e.*, rotating the plane of polarization) must be optically active bodies themselves (this includes nearly all except

the fat-splitting enzymes). They act like catalyzers, attach themselves to substrate and make a new body with it which stands higher temperature than ferment alone. Trypsin may distribute itself between several different kinds of proteids if such are present at the same time, and then the proteins of more difficult digestibility act as inhibiting substances to the ferment. (Trypsin in a solution of casein, fibrin and egg albumen.) This gave rise to the idea of anti-ferments in such solutions.

Laws of manner of effect of ferments are not known because they have not been prepared in pure state. Hydrolytic ferments cause no changes in temperature and hence they cannot be claimed to act as catalyzers, *i.e.*, simply accelerating a process already started. Sugar, proteins and peptones can be kept for years without change, but undergo cleavage as soon as they come in contact with the proper ferments, these therefore initiate the process, hence the ferments cannot be claimed to accelerate a process already begun (Conheim, *Physiol. d. Verdauung*, p. 107).

The beginning of ferment action is extremely rapid. Sugar can be detected in starch almost immediately when saliva touches. There is a glycolytic ferment in muscle which forms $CO_2$ explosively from glucose after it comes together with an activator produced in the pancreas. The rapid beginning is a constant sign if the enzymes are allowed to work under the correct conditions. But in experimental conditions this action ceases quickly also. No matter how much glucose is present, the glycolytic action and $CO_2$ formation ceases in 2 to 10 hours. Explained 1, by the fact that the ferment may be used up; 2, or destroyed because labile; 3, or inhibited by its own products.

A constant characteristic then, is quick onset, and quick cessation.

Ferments and Temperature and Reaction. The enzymatic effect is only slight in the cold. Organic enzymes are best adjusted to a temperature of 30° to 37° C.

*The Hydrolytic Action of Diastase on Starch.*—Five grams of crushed germinating barley together with 10 grams of potato starch and 20 c.c. of cold water are placed in a Florence flask; now add 70 c.c. of hot water while constantly stirring the mixture.

Put the flask on a water-bath and keep at about 60° C. for one hour. Now taste the liquid. Does it taste sweet? Make a note. Heat 10 c.c. of Fehling solution to boiling and add drop by drop some of the diluted and filtered starch solution. A red or yellow precipitate is formed and is either cuprous oxide or the yellow hydrate of copper which has been reduced from the copper sulphate in Fehling's solution.

Some ferment which has been secreted by the cells in the germinating barley has caused the starch molecule to take up molecules of water. The starch has been converted by hydrolysis into sugar. Fehling's solution reduces sugar but has no effect on starch.

*Separation of Diastase from Germinating Barley.*—Diastase is a product which is formed in living cells and is brought about by the life activities of the barley cells. It is a ferment which can cause the hydrolysis of starch. This can be proved by the following experiment: Add to germinating barley half its weight of water, macerate thoroughly and heat over a water-bath at a temperature of 35° to 40° C. for two hours. Place the mixture in a linen cloth and press out the water extract. To this an excess of alcohol is added and there will be a precipitation of diastase. This can be further purified by dissolving in water and again precipitating with alcohol. The exact nature of this chemical compound is not known.

Iodine colors starch blue. A blue color does not result when iodine is added to sugar. Make 10 c.c. of starch paste, and add a little diastase; when this mixture is tested immediately a blue color will result. Later the starch will be converted into sugar and the blue color will not be obtained.

*The Hydrolysis of Starch by Salivary Diastase.*—A test-tube containing 2 c.c. of filtered saliva and 10 c.c. of starch paste is kept at a temperature of 35° to 40° C. for about fifteen minutes.

Now test with iodine for starch. Is starch still present? Test for sugar with Fehling's solution. The solution which at ·st was opalescent, later became clear; this was a purely physical nge. Later, test again with iodine; a red color indicates the

presence of erythrodextrin. If the color is violet some unchanged starch is still present.

Again test at a later period with Fehling's solution. If there is no change in color produced it is because erythrodextrin has been converted into another dextrin, called achrodextrin. Achrodextrin gives no change of color with iodine. At this stage the presence of maltose may be shown by testing with Fehling's solution. Still another sugar, dextrin, is present and it may be differentiated from maltose by the aid of the polariscope. Dextrin rotates the plane of polarization less than maltose does.

*The Dialysis of Sugar.*—A starch solution to which some saliva has been added is incubated for twenty hours in a parchment-paper dialyzer tube. Test for the presence of starch and sugar in the water surrounding the dialyzer. The test for sugar will be positive; that for starch negative.

During the digestion of starch a whole series of dextrins is probably formed. The starch is not converted directly into sugar. Some of the dextrins which are formed may appear only as forerunners of the sugars, while others appear merely as concomitants of its production. Some of the latter probably are never converted into sugar, and sugar may appear when only a small portion of the starch has been converted into achrodextrin. Achrodextrin is partly, but never completely converted into maltose in artificial digestion, and at the end can be precipitated by the addition of alcohol to the liquid. If the sugar is removed as it is formed the residue of unchanged dextrin is less than when the sugar is allowed to remain. Under favorable circumstances in ordinary artificial digestion, from 12 to 25 per cent. of the starch is dextrin, but the residue of dextrin may not be more than 4 per cent. in dialyzer digestions. The dialyzer digestion more closely approximates what takes place in the alimentary canal, where the digestion is exhaustive and complete. Both starch and dextrin are found in the stomach after the ingestion of starch, but in the intestines only minute traces are found.

*The Selective Action of Ferments.*—Place a small piece of

fibrin in several centimeters of filtered saliva and keep at a temperature of 37° C. for two hours, make a biuret test and notice that no change has occurred in the fibrin.

Repeat the above experiment, using however, 0.5 c.c. of neutral olive oil. Again no consequential changes are noted.

The ptyalin ferment action is in a way "specific," and has a decomposing action only on carbohydrate on starch and does not decompose proteids, fats or oils. All ferments act specifically in a similar manner.

## Preparatory Experiments for Study of Absorption and Secretion.

*Semi-permeable Artificial Membranes.*—A gram molecule of any substance is the quantity in grams of that substance equal to its molecular weight. A gram molecular solution of any substance is one which contains a gram molecule of the substance per liter. Thus, a gram molecular solution of sodium chloride is one which contains 58.46 grams NaCl.

Na = 23.00, Cl = 35.46 in a liter. The letter "M" stands for *molecular* in the formulæ.

Prepare the following solutions:

1. $\frac{M}{5}$ (5 per cent.) $CuSO_4$.

2. Two per cent. $\frac{(M)}{20}$ $K_4Fe(Cn)_6$.

3. Ten per cent. gelatin solution, boiling it till it no longer gelatinizes.

4. Make a solution of equal parts of M cane sugar solution and 5 per cent. tannic acid: Color part of this solution with Congo red.

All substances in solution tend to diffuse from regions of a higher to those of a lower concentration. The energy to which this movement is due is *osmotic pressure.*

If solution and solvent are separated by a membrane which is permeable to the solvent but not to the dissolved substance, the effect of osmotic pressure is seen in an increase of the volume of

the solution, due to the passage of the solvent into it through the membrane.

Semi-permeable membranes are of universal occurrence in living organisms, and are represented by the cell walls of animal cells and the plasma-membranes of plant cells.

EXPERIMENTS.—1. Using a fine-pointed pipette, introduce a drop of $\frac{M}{5}$ $CuSO_4$ solution beneath the surface of a 2 per cent. $\frac{M}{20}$ $K_4Fe(Cn)_6$ solution in a watch glass.

Do the solutions mix? Why? Note the size of the $CuSO_4$ drop; set aside and note if its size changes in an hour.

2. Ascertain if the semi-permeable membrane of $Cu_2Fe(Cn)_6$ is impermeable to other substances, *e.g.*, sugar. Inject a globule of $CuSO_4$ solution with a drop of $\frac{M}{2}$ solution of cane-sugar using a fine long-nozzled pipette.

What becomes of the sugar solution?

Does the sugar solution flow out?

What do you conclude?

3. Introduce a drop of equal parts of 5 per cent. tannic acid and M cane-sugar solution beneath the surface of a 10 per cent. gelatin solution in a watch glass.

What is the character of the membrane formed?

Use some of the Congo red colored solution in another dish of gelatin.

Try tannic acid alone without the M cane-sugar. Is there any difference? Why?

Does the Congo red escape from the globules? Do the globules change in size after an hour? Is the membrane permeable?

A finger parchment is tied tightly over a rubber cork. Through the center of this cork a glass tube of 3 to 4 mm. inside bore is tightly fitted. The tube may be several yards long. This whole arrangement is held in a beaker from an iron stand.

Fill the beakers of three osmometers with water. The finger

parchments shoud be filled, one with a $\frac{M}{4}$ cane-sugar solution, another with $\frac{M}{4}$ NaCl solution and the last with a gelatin solution: Osmosis of $H_2O$ will slowly occur in each case from the water in the beaker into the solution. As a consequence the columns of fluid in the tubes will rise.

Note the height of the fluid in each tube after twenty-four hours, and after forty-eight hours.[1]

## Demonstration of a Dog with an Experimental Accessory Stomach.

The demonstrator will perform a Pawlow operation upon a dog or an animal in which the esophagus will be severed and the ends stitched to the outside of its body. (For technics refer to J. P. Pawlow in *Ergebnisse d. Physiol. Jahrg.*, 1, p. 246. (See Appendix.)

*Fictitious Meal for Stimulation of Gastric Secretion.*—Feed a dog with esophageal fistula and gastric fistula by giving him bread to chew. The swallowed bread will fall out at the esophageal fistula, but gastric juice will drop from the canula in the the stomach although no food enters this organ.

*Psychic Gastric Secretion.*—A cannula will be inserted into the dog's stomach and food will be placed so that the dog can see and smell it. Gastric juice may be collected from the cannula. Although the animal does not even taste the food.

On another day place some boiled beef directly into the stomach of the dog, diverting his attention by petting him, so that it is done without the knowledge of the dog or animal that it is being fed. Notice if there is any difference in the flow of the gastric juice. An extract of the salivary glands is injected into the peritoneum. Notice what effect this procedure may bring about on the flow of gastric secretion. (For effect of salivary gland extracts on gastric secretion see Hemmeter in *Biochem. Zeitschr. Hamburger Festschrift*, 1908, p. 238.

[1] A conference should follow on the physical forces concerned in absorption and secretion, for example, osmosis, dialysis, diffusion, filtration.

That saline extracts of salivary glands when injected into a vein or peritoneum can produce secretion of gastric juice has been confirmed by Th. Mironescu (*International Beiträge z. Path. Therap. d. Ernährungstör, Stoffmechsel u. Verdaungskrank*, Bd. I, p. 195, and also by Otto Emsmann, *ibid.*, Bd. III, 1911, p. 118.

Inject a 0.01 per cent. solution of belladonna, pilocarpine and morphine into the peritoneum and note the effect of each on the gastric secretion.

## The Preparation of Artificial Gastric Juice.

1. A portion of mucous membrane which has been stripped from the fourth stomach of a calf is immersed in cold water until there is no further acid reaction and is then dried in the air and divided into small pieces. Dilute hydrochloric acid is then added.

2. The mucous membrane is removed from the deep layers of the stomach of a pig or a rabbit and is cut into small pieces, which are thoroughly washed with water. The moist residue is carefully preserved and covered with glycerine. It is necessary to add dilute hydrochloric acid before using. Make a dilute solution of hydrochloric acid by adding 10 c.c. of officinal HCl, sp. gr. 1.124 (about 25 per cent. HCl) to enough water to make 1000 c.c.

*The Digestive Action of the Pepsin in the Artificial Juice.*—Place a weighed amount of fibrin or meat in each of three flasks; label the flasks A, B and C. To A add 100 c.c. of the artificial gastric juice; in B put 100 c.c. of a 0.2 per cent. solution of HCl, and in C introduce 100 c.c. of distilled water, and a piece of dried gastric membrane. The three flasks are kept at a temperature of 37° for five hours. It will be noticed that the artificial gastric juice in flask A has digested the fibrin or meat, but that the hydrochloric acid solution in flask B has not done so. In flask C notice if any digestion has taken place.

*Estimation of the Quantitative Effects of the Constituents of Gastric Juice.*—(Pepsin and hydrochloric acid.) The Comparative Digestorium.

The following modification of Ewald's four test-tube method has given much more useful and available results in my laboratory. I use a large glass trough about 10 to 12 inches long and about 8 inches high. A very large glass beaker will answer the purpose equally as well, but it may not be possible to suspend as many parchment digesting tubes in it, as in the long trough which I recommend. From glass rods resting across the top of the trough or beaker six parchment tubes are suspended, each about three-fourths of an inch in diameter, The trough has an

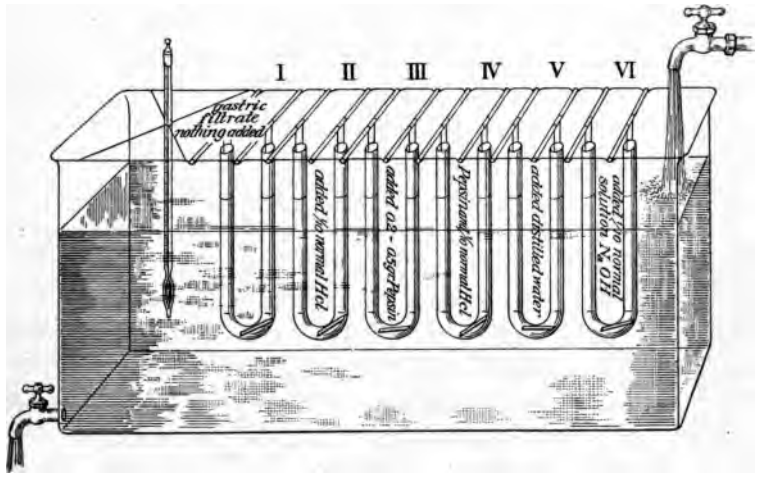



FIG. 49.—Authors Comparative Digestorium Explained in the Text.

outflow tube at the lower edge at one side. It is not necessary to change the water often if no more than 5 c.c. of gastric juice is used. In that case smaller membranous tubes only half an inch in diameter, will suffice. In each of the six membranes suspended in the trough in this manner we pour 5 c.c. gastric juice, and also add one Mett tube or a weighed amount of crystallized serum albumen. (Ewald used disks of hard-boiled egg white about the size of shirt buttons.) Now the tubes are numbered from left to right from 1 to 6. To tube No. 1 nothing is added but the gastric juice and the serum albumen or Mett tube. To tube No. 2 a decinormal solution of HCl is added, drop by drop. If by a previous

test with phloro-glucin-vanillin free HCl has been found entirely absent, or by titration the HCl has been found deficient, it should be added until it gives the reaction for free HCl with Congo red. To tube No. 3 there is added 0.2 to 0.5 grams crystallized pepsin. To tube No. 4 pepsin plus HCl. To tube No. 5 nothing is added but distilled water, diluting the contents as follows: 1c.c. gastric juice with from 1 to 10 c.c. of distilled water as may be necessary. To tube No. 6 we add 1.10 deci-normal solution of sodium hydroxide, but only in case a previous titration has demonstrated that the HCl is present in excessive amount. The amount of deci-normal NaOH added should correspond to the amount of excess of HCl above the normal. This digestorium with its six parchment tubes is placed in an incubator with the thermostat registering about 98.6° F. The period for the digestive process for Mett's tubes is about ten hours. The digestorium can be made larger to contain several tubes of each kind, so that a tube can be removed after varying periods of time have been allowed to elapse, in order to study the effect of time on the degree of proteolysis. We next ascertain in which parchment the Mett tube has been most digested. While the process is going on, the rapidity of the digestion of the Mett tubes or of the albumen disks, or of the crystallized serum albumen, will inform us whether proteolysis would have occurred without having added anything. The Mett tube in the parchment dialyzer No. 1 should have shown a normal number of millimeters of digestion solution of the albumen column (from 2.5 to 3 mm.). If it is not dissolved in No. 1, but in No. 2, this would indicate that the gastric juice was deficient in HCl, and a further titration with HCl will be necessary to inform us of the amount of the deficit of HCl. If it did not digest in dialyzer No. 2, but in No. 3, it would indicate that pepsin was lacking, and additional tests with gradually increased additions of crystallized pepsin, will be necessary to inform us of the pepsin deficit. If only the tube, in dialyzer No. 4, shows a proper digestion, it would indicate that both pepsin and HCl were lacking; whereas, if only dialyzer No. 5 showed a normal pro-

teolysis of the Mett albumen tube, it would indicate that that gastric juice as it was drawn from the stomach already contained too great a concentration either of salts, or of the product of pepsin digestion. It is in this dialyzer No. 5 that overactive gastric juices can best be detected; and if the arrest of proteolysis was due to an excess of HCl, the best digestion of Mett tube of albumen will be found in Mett tube No. 6.

The advantage of parchment tubes over glass test-tubes consists in the removal by dialysis of the end-products of peptic digestion, which, as is well known, arrest the proteolytic process by their accumulation.

*Extraction of Pepsin.*—The following method may be used to extract pepsin. Mucous membrane from the stomach of the animal selected is acidulated with phosphoric acid, and is allowed to digest until most of the proteids are converted into soluble peptone. Neutralize the mixture with lime water and insoluble calcium phosphate is formed. This settles to the bottom of the vessel and carries down with it the pepsin. Very dilute hydrochloric acid is used to dissolve this precipitate. A solution of cholesterin in alcohol and ether is now added. On mixing these solutions cholesterin separates as an abundant fine powder bearing the pepsin with it. The pepsin is recovered by removing the cholesterin with ether. A mechanical precipitant which is sometimes used is ammonium sulphate. The author has found that aluminium silicate acts in a similar manner.

### Proteid Converted into Peptone by Pepsin.

Make a glycerin extract of pepsin and add 5 c.c. of a 0.2 per cent. hydrochloric acid solution, to 5 drops of the glycerin extract. Put in a small piece of fibrin and keep at a temperature of 35° to 40° C. Notice how long a time passes before the fibrin is dissolved. Test for albumoses and peptones in the following manner: A saturated solution of ammonium sulphate is added, and the albumoses are precipitated. Filter. Add to some of the filtrate in a test-tube 2 drops of a very dilute solution of cupric sulphate. Now add sodium hydroxide in excess; a pink color will develop; this is the biuret reaction.

## Action of Rennin on Casein.

A rennin extract is made as follows: Treat some mucous membrane of the fourth stomach of a very young calf with 150 to 200 c.c. of 0.2 per cent. hydrochloric acid solution for twenty-four hours. Then neutralize the acid with great care. The extract which we have prepared contains both pepsin and rennin. The object is to isolate the rennin. This is done as follows: Shake the neutralized extract repeatedly with fresh amounts of magnesium carbonate; the precipitates which are formed will carry down little of the rennin but most of the pepsin. The filtrate coagulates milk promptly, but contains only traces of pepsin. The filtrate is now precipitated with lead acetate; then is decomposed with very dilute sulphuric acid and the mixture is filtered. The filtrate contains the rennin and to it is added a solution of stearin soap in water. This causes the soap to be thrown out of solution and it falls, carrying the rennin with it. Shaking with ether will remove the soap and leave the rennin. According to Pawlow rennin [or chymosin] and pepsin are one and the same enzyme—manifesting itself in two different effects. According to researches of the Author they are separate and distinct enzymes (Hemmeter in Journ. A. M. A., Dec. 9, 1905, also in Berliner Klinish, Wochschr.) Ewald Fest heft. 1905.)

## Precipitation of Casein.

Add 1 c.c. of neutral rennin to 25 c.c. of fresh neutral milk at a temperature of 36° to 38° C. After the curd and the whey have separated the curdled portion which consists of casein together with the fat globules is placed to one side. The whey is a dilute saline solution containing milk albumen, milk sugar, etc. It will be found that the mixture is still neutral. Curdle some milk by adding an acid. It must be noted that although curdling occurred in the first part of the experiment, there was no acid reaction observed. Milk can also be curdled by lactic acid fermentation.

The following experiment will explain the action of rennin:

*Experiments of Arthus and Pages.*—Prepare two solutions having the following contents:

No. 1. Milk, 100 c.c.
Neutral oxalate of potassium 1 per cent., 5 c.c.
Rennin, 1 to 250=4 c.c.

No. 2. Milk, 100 c.c.
Neutral oxalate of potassium 1 per cent., 5 c.c.
Water, 4 c.c.

These two solutions are similar except that one contains rennin and the other does not. Both mixtures are kept at 38° C. for forty minutes. If 25 c.c. of each solution be boiled, the one which contains the rennin will coagulate, while the one that does not, remains unchanged with regard to its fluidity. The casein has been rendered coagulable by the action of the rennin. Now take 25 c.c. from each stock solution and add calcium chloride until no further precipitate is obtained on addition. Potassium oxalate is removed by this procedure and the calcium chloride remains in excess. Equi-molecular selections should be used.

Solution 1 will coagulate on boiling but solution 2 will not, because the casein in solution 1 has been precipitated by the addition of a small quantity of calcium chloride.

If, however, 5 c.c. a 1 per cent. of calcium chloride solution is added to solution No. 1, this will exactly combine with the 5 c.c. of potassium oxalate and No. 1 solution may also be precipitated in this way.

Rennin causes coagulation in greater amounts the longer it is allowed to act. This may be tested by adding small quantities of rennin to neutral milk, and equal portions of the milk be tested by boiling from time to time.

An amount of calcium chloride which may have been too small to produce coagulation in the early stages of rennin reaction, may be sufficient to produce coagulation when added in the later stages. Two separate phenomena may be distinguished in the clotting of milk: 1. Is a chemical transformation of casein by

rennin; 2. transformed casein is precipitated by the calcium salt, A. S. Loevenhart (Hoppe–Seyler, Zeitschr. physiol. Chemie Bd. 41. p. 177. 1904) suggests that oxalates, citrates and similar salts and certain acids play a rôle in milk coagulation in the following manner, namely they simply set free calcium, and Jacques Loeb agrees with this view and suggests that the *Rennin* in the coagulation of milk seems solely concerned in rendering available calcium which naturally is held in organic combination in the milk in such a form as to be of no use for the process of coagulation. (Dynamics of living matter p. 91). Compare this effect and the rôle of calcium in blood coagulation. What is the difference between the two types of coagulation with regard to calcium?

## Precipitation of Fibrin by Fibrin Ferment.

Secure 100 c.c. blood serum by straining a blood clot through a linen cloth, and add 10 c.c of this serum to 10 c.c. serous transudate, such as ascitic fluid, pleural effusion, or hydrocele fluid. In a few hours a firm, translucent clot will form.

*Gamgee's Method of Extracting Fibrin Ferment.*—Wash a blood clot free from its corpuscles and the fibrin which is thus freshly secured is allowed to stand for several days in an 8 per cent. solution of sodium chloride. Then filter.

The filtrate is very rich in fibrin ferment.

## To Extract Fibrinogen.

Arterial blood is drawn directly from an artery into a vessel containing a saturated solution of magnesium sulphate. The magnesium sulphate is to prevent clotting and should equal in volume one-third the amount of blood drawn. The plasma can now be separated from the corpuscles by centrifugalizing. Add to the plasma an equal volume of a saturated solution of NaCl. The flaky precipitate is fibrinogen. Several funnels are now prepared and the precipitate is collected on the filter-paper. Small pieces of the filter-paper containing the fibrinogen are placed in a quantity of 8 per cent. sodium chloride solution

equal to about one-third the quantity of the magnesium sulphate solution which was originally used to prevent clotting. The fragments of paper are removed by filtering. An equal volume of saturated sodium chloride solution is added. Filter and dry between filter-paper and add a small quantity of water to the finely divided filter to which the precipitate clings. This water will remove a small quantity of salt from the precipitate and the fibrinogen will dissolve in this dilute saline solution.

*Precipitation of Fibrinogen by Fibrin Ferment.*—If a solution containing fibrin ferment is added to the dilute solution of fibrinogen, there will be a gradual formation of fibrin.

## Ammoniacal Fermentation of Urea.

Take 10 c.c. of urine which has undergone ammoniacal fermentation, add 50 c.c. of 95 per cent. alcohol and stand aside for five days in a well-corked flask. Now filter through a small filter and wash the precipitate with fresh alcohol.

The precipitate contains cells of yeast and numerous bacteria. Add a small quantity of the precipitate to a neutral 2 per cent. solution of urea and place the mixture over a water bath at 38° C. Test the reaction. Notice the odor of ammonia which appears in a short time.

## Extraction of Lipase.

The pancreas from a recently killed pig should be freed from fat as much as possible, and ground up in a mortar with quartz or with coarse, clean white sand. Extract the lipase with a small amount of water. Lipase can be extracted from the liver of the pig. The liver from a recently killed pig is ground to a fine pulp in a mortar with about 200 c.c. of water. Filter, and dilute the watery extract to 500 c.c.

### The Hydrolytic Action of Lipase on Ethyl Butyrate.

Place in each of two test-tubes, A and B, 1/10 c.c. toluene, 4 c.c. water, and 0.26 c.c. of ethyl butyrate.

Cork the tubes tightly. Bring them to the temperature of 40°

C. by placing them in a water-bath. To each of the tubes add 1 c.c. of the aqueous extract of lipase. Boil tube b. Let both tubes remain at 40° C. for fifteen minutes. Remove them from the bath. Plunge the tubes into ice water to check further fermentation. Titrate with N/20 KOH, using neutral red as the indicator. The initial acidity of the enzyme solution, usually 0.1 to 0.2 c.c. N/20 KOH, should be deducted from the cubic centimeters of KOH required to neutralize the fatty acid formed. Fatty acid will appear in tube A, but not in tube B, in which the enzyme will be destroyed by boiling. Loevenhart and Kastle (*Am. Chemic. J.*, 24, 491, 1900) have demonstrated that the action of lipase is reversible, *i.e.*, it can split up ethyl butyrate into butyric acid and alcohol, but also resynthesize these two into ethyl butyrate. Perform above experiment taking butyric acid and alcohol and add lipase test as before.

## Relation of the Glycolysis to the Pancreas and the Lymph.

A dog is made to fast for thirty-six hours, the amount of sugar ascertained in the urine, and then anesthetized, and the pancreas removed. Then again determine the amount of sugar present in the urine at intervals of a few hours. The amount of sugar occurs in increasing quantities and may rise even as high as 20 per cent.

Feed a second dog upon a liter of milk and in two hours withdraw from its thoracic duct 15 to 20 c.c. of lymph and inject this lymph into the jugular vein of the dog from which the pancreas has been removed.

The glycosuria will greatly diminish at first, but after a few hours it will become more intense and will continue until death. A greater quantity of sugar will also be found in the blood than normal.

These phenomena are due to the disturbance of interaction of two ferments; or rather, one ferment—the glycase of muscle, and its activator, which comes from the pancreas. (*O. Cohnheim, Zeitschor f. physiol. Chemie*, 39, 396 (1903); also 42, 401 (1904); 43,

p. 547 (1905). The activator is supposed to be contained in the lymph of the thoracic duct of normal dogs, being absorbed during digestion.

### Extraction of Glycase.

The pancreas which has been aseptically removed is crushed at once in 100 c.c. of sterile water containing 0.2 gram sulphuric acid. After macerating for two hours at 38° C. neutralize with NaOH and add 0.5 gram of pure glucose. The mixture is now kept at a temperature of 38° for one hour.

Estimate the amount of sugar. It will be found that the loss will range from 20 to 40 per cent.

A much less loss of sugar occurs when the pancreatic extract is made without sugar. Hydrolysis probably causes the production of the glycolytic ferment from a zymogen.

A glycolytic power is acquired by malt diastase, or salivary diastase which has been kept for three hours at a temperature of 38° C. in water containing 0.1 per cent. of sulphuric acid. But malt diastase or salivary diastase can change starch into sugar in the absence of a weak acid.

A glycolytic power occurs in pancreatic juice to which a small amount of dilute acid has been added. The amylolytic power is soon lost in the juice as obtained in the laboratory. The above experiments show that oxidizing ferments are widely distributed. They are found in plants as well as in the blood, pancreas, liver and lymph of animals. They are present also in the urine and generally known as *oxidases*. Pancreatic extract should be studied in the various admixtures in the comparative digestorium, p. 144.

## CHAPTER XII.

### Internal Secretions.

As far as I was able to ascertain the term *internal secretion* was first used by Claude Bernard in 1855; *Leçons de Physiologie Experimentale*. He applied this term to the glycogenic function of the liver which he called "*sécrétion interne*" while he designated the secretion of bile as "*sécrétion externe*." Curiously

enough, the glycogenic function of the liver is not nowadays classed strictly among the internal secretions but regarded as a special provision for the adaptive storage of food material. Since Claude Bernard's publication an enormous amount of literature has accumulated on this subject, in much of which there is an inclination to unjustified conclusions; many physiological processes that were imperfectly understood were prematurely classified under internal secretions. In all living things whether they are unicellular or multicellular, the living protoplasm forms various substances as a result of the activities of metabolism. The fate of these substances may be one of three varieties: 1. They are rejected as refuse material and secreted into the media that surround the living thing. 2. They are utilized to transform and aid in the absorption of nutritive material (external secretions of the digestive canal). 3. They serve some important purpose in the economy or regulation of function of the cell that produced them or in the more complex organisms, of other cells (internal secretions).

In order to understand the term internal secretion it is desirable that the concept *secretion* should be understood and clarified. The word *secretion* as it is generally used implies the separation from the blood or lymph of one or more substances through the activity of the living protoplasm of cells. Johannes Müller, who was not only a brilliant experimentalist, but also a profound philosopher, conceived the secretory process to consist of two phases: 1. The production of certain substances; 2. the separation of these substances at a surface either within or outside of the body. The first phase he called *secretion*, the second *excretion*. The excreted substance could in some instances be previously found in the blood stream; it was then simply removed toward the exterior; by the tissues of an organ, this applies to the case of urea, which was considered by him a pure excretion. Although a substance may be a waste product and of no further use to the organism and thereby deserve the term "*excretion*," yet the process by which it is removed involves the secretory activity of certain cells; thus, while *urea, the sub-*

*stance* is in itself an *Excretion*, the process of the renal cells by which urea is removed is a *Secretion*. Thus, the expressions *Excretion* and *Secretion* occasionally cover each other in part. When a metabolic product is of no more use in the economy of the organism it is an excretion. The activities and effects on the secretory organs are twofold: 1. The separation from the blood of substances which previously existed in the blood, for example, urea which is removed by the kidneys, also lactic acid and its salts; these are excretions. 2. Substances which are not simply removed from the blood since they do not exist in it previously but on the contrary are formed anew from similar constituents of the blood by a chemical process; for example, mucus, milk, bile, the various enzymes; these are the secretions. The characteristic of all external secretions is that they are poured out on a free surface of the body, but the characteristic of internal secretions is that they are given off into the blood stream. The definition of internal secretion is therefore the following: *the elaboration of specific, chemical substances out of a raw material furnished by the circulating blood, by definite and typical gland cells; these substances are not given off on a free surface but into the blood stream.*

Living things belonging to the class of multicellular organisms are most complex laboratories, in which chemical and physical energies are active in the most manifold and divergent ways.

It is therefore necessary that there should be effective connection, communication, co-ordination and reciprocal adaptation between these many types of form and function. Thus far we know of two methods by which this co-ordination is affected: 1. by the central nervous system; 2. by the intervention of chemic agents with which internal secretion has to do. The co-ordination of different parts of an organism for a congregate purpose exists in all living things, but a nerve system is only found in the higher metazoa, and in the absence of a nervous system the only other possible means of co-ordination is through chemical agents distributed through the blood or lymph or both. In the unicellular organisms, for example bacteria-protozoa, the only adaptations

that we can gain insight into are those to the environment of these organisms, and in that case the mechanism is one of osmosis and dialysis; in short, a chemical mechanism. These organisms approach their food or flee from injurious influences or seek the light as a result of chemical stimulation. They prepare their digestive nourishment by chemic substances, viz., enzymes, and they defend themselves by chemic substances, viz., toxines. As we have seen in the practical exercises on direct observation of the circulation, the accumulation of phagocytic cells around a foreign body is brought about by chemic stimulations that emanate from all damaged tissues. The phagocytes of the higher organisms resemble the monocellular organisms in their conduct —tropisms and chemic orientation. Even in the lower metazoa, for example, the sponges, there is no trace of any nervous system, the co-ordination between the various cells of the colony is determined by purely chemical means.

As soon as a central nervous system appears in the ascending scale of metazoa, the prompt and rapid motor or secretory (chemic) reactions of this system predominate to such an extent in the life manifestations of the animal that they almost conceal or at least push into the background, the chemic co-ordinations which really preceded them—phylogenetically.

The essential difference between the two types of correlation is one of time. The nerve system was developed for the purpose of quick adaptations and reactions, not as might be imagined, for the purpose of abolishing the slower chemic correlations which existed before a nerve system was formed.

When a reaction occupies seconds or fractions of a second, the nervous system must necessarily be employed; but where the reaction requires minutes, hours, or even days, the correlation between the organs must be regarded as a chemical one. In many instances we have been enabled to demonstrate the existence of such a chemical connection and been able to reproduce experimentally, conditions of growth and function which under normal conditions constituted only one phase of a complicated series of physiologic events.

The chemical reactions and co-ordinations of the organism as well as those which are adaptations by intervention of the central nervous system may be divided into two grade classes.

1. Those reactions and adaptations which are produced in consequence of changes forced upon the organism as a totality from *external* causes. This class includes the reactions to chemical poisons generated by bacteria or higher organisms that have invaded the body and they represent an important means of the body to defend itself in the struggle for existence. All the protective substances referred to in the modern theories on immunity belong to this class.

2. Those chemical reactions and adaptations which act entirely *within* the body, and whose purpose is to bring into correlation the various parts and organs of the body in the most extensive manner. This correlation of the various tissues and organs of the body is brought about by the internal secretions which contain definite and specific chemical substances that serve as messengers between these various structures. As it was of convenience to have a name for these chemical messengers, W. M. Bayliss and E. H. Starling suggested the term "hormone," from the Greek ὁρμαω, "to excite, awaken, or stimulate." The name is well applied to the majority of these substances thus far studied, but as some of them do not stimulate, excite, or awaken, but rather inhibit and check, I have suggested a second term for those chemical messengers that are opposite in their effect to the hormones and named them "koliones," from the Greek ψωλυων, "to check, to arrest, to inhibit." (See Hemmeter, Proceedings of the Society for Experimental Biology and Medicine, Vol. vi, 1909, p. 33–44.)

Both hormones as well as koliones can be considered originally to have been bi-products of a specific function of the organs that produced them; that means, they were not the chief product of that organ but accidental, secondary products. The next stage in the development of a correlation was the acquirement of a sensitiveness to these accidental substances in any remote organ, especially such an organ that was functionally related to the one

that produced the hormone. (See Bayliss and Starling, *Zentralbl. f. d. gesamt. Physiol. u. Pathol. d. Stoffwechsels*, N. F., Bd. II, p. 164).

In the foregoing I have perhaps held apart neurogenic and chemogenic co-ordination and correlation too strictly. It is possible that instead of being separate and distinct, these two methods of correlation may simply be different phases of the same process, or they may supplement each other in a reciprocal manner, for we already know of one hormone, the best known, that occurring in the adrenal bodies and called *epinephrin* or *adrenalin*, that it does not act on any tissue or organ that has no sympathetic or autonomic nerve supply; in fact its point of attack seems to be the end arborization of the nerve fiber where it unites with the muscle, the synapse (Langley), and the presence of adrenalin in the body appears to be a necessary condition for the normal functioning of the entire autonomic system by means of reflexes.

It must be evident from what has been stated in the preceding, in the definition of secretion and internal secretion, that all definitely morphologic elements like cells, especially lymph cells, spermatozoa and ova are excluded from the external secretions and cells produced by the spleen, bone marrow and lymph glands are excluded from the internal secretion. The internal secretions are best considered under three hearings: *First*, internal secretions of those organs which have also an external secretion. These are the liver, pancreas, kidney, the intestine and the stomach glands. *Second*, the internal secretion of the organs of generation, the ovaries and testicles. *Third*, the internal secretion of the glands without excretory ducts, namely, the adrenal bodies, the thyroid, the parathyroid, the hypophysis, consisting of (a) infundibulum or nervous part, and (b) the glandular part, and finally the thymus glands. There are a number of glandular bodies which have been omitted in this schema. They surround the carotid artery or they are in and around the conduits of the sympathetic nerves; a special one is the pineal gland and the coccygeal gland and are usually referred to as the

Chromaffin cell groups, from the acidity with which they take up certain pigments or stains.

## Laboratory Exercises with Internal Secretions.

A number of these internal secretions do not lend themselves for practical experimentation in the laboratory because their preparation requires skill and experience and involves too much time and in fact the so-called internal secretions vary so much with the general functional activity of the organ that no dependence can be placed on the method of preparation. However a few of the most instructive operations and exercises will be given in the following. They are studied in six different manners or methods: 1. A definite organ is removed from the body under observation of the strictest surgical technic and asepsis; thereafter the effects on the general organism are studied. 2. The reverse of this, the implantation of an organ or gland or tissue into another intact organism and subsequent study of the effects. 3. Feeding of fresh organs or tissues or extracts therefrom (opotherapy). 4. Subcutaneous injections of extracts of organs or tissues either fresh or prepared according to different methods. 5. Intraperitoneal injections. 6. Intravenous injection.

## Effect of Organ Extracts on the Blood Pressure.

All animal tissues and organs give off substances to watery or salt solutions which influence blood pressure, especially if the salt solution is closely similar in the contents of salts to those contained in the normal plasma of the animal. All of these extracted substances exert a depressor effect on the blood-vessels excepting two; the extracts of the medullary portion of the adrenal body and of the infundibular part of the hypophysis cerebri or pituitary gland which cause a rise; but it must not be thought that the presence or absence of either the "*pressor*" or "*depressor*" substance is a necessary evidence of an internal secretion in the organ that yields it.

## Methods of Preparation.

The organs, glands, cells or mucous membranes are either ground up or rubbed up in a mortar with quartz and then extracted either with normal salt solution or Ringer's solution. If the extracts are to be used at once, the filtered salt solution sufficies. In this way the tissues give up not only the possible hormones, but all extractive substances, like ferments, albumens, neucleic acid, carbohydrates, lecithin-proteid combinations. If the salt water extracts are kept feebly alkaline and used within two or three hours, they are perfectly servicable for the *practicum*. I do not advise the perfect purification as practised by M. Jacobi, or the entire removal of proteids, according to Brücke, for the student exercises. It is advisable to obtain the extracts of the adrenals of the hypophysis and of the thyroid and any other organ extracts that can be obtained from a reliable manufacturer.

## Internal Secretion of the Suprarenal Glands.

In order to give to the student at least one example of the necessary care and skill involved in studying internal secretions by one of the foregoing methods, it is recommended that the demonstrator operate a large Belgian hare, removing the suprarenal glands in the presence of the class. They will be impressed with the fact that to save the life of this animal it is not possible to remove both adrenals on the same day. Two weeks must intervene between the removal of the two adrenal bodies.

**Operative Directions for Removal of First or Left Adrenal Gland.**—Starve the hare for twenty-four hours; inject 10 centigrams of morphine sulphate under the skin; tie the animal, back down, upon the Harvard rabbit board; wash the abdomen with hot water and soap, then with bichloride of mercury and shave the hair from the middle portion of the abdomen. Use strict antiseptic technic throughout. The left suprarenal must be removed through the abdomen; the right one can be removed two weeks later entering through the back of the animal, which is not so dangerous a procedure. Make an incision about three

inches long through the median, linea alba. Beginning at the xyphoid cartilage, tie any small cutaneous vessels that bleed, or catch them with a small bulldog forceps. The hands and forearms of any assistants or students that aid in the operation must be sterilized as for a major operation on a human being. After the incision is carefully extended down to the peritoneum, pick this up with a fine forceps, then make a small cut into the part thus picked up and enlarge the cut through the peritoneum to the size of the skin wound. Sterilized absorbent cotton pads must be ready, wrung out in hot sterile salt solution. An assistant holds back the edges of the abdominal wound with retractors. The intestines are covered up with the warm cotton pads. Insert the hands into the abdominal cavity and feel for the left kidney. Locate the vein of this kidney and follow it to its junction with the inferior vena cava. Closely hugging the latter vessel and in the angle formed by these two veins, the left suprarenal body will usually be found. It is underneath and behind the peritoneum which has to be torn through by a blunt seeker; a strong light is necessary for this part of the operation. The gland is separated cautiously from its surrounding tissue and removed. It is necessary to avoid injury to the vena cava.

With a view to orientating for the removal of a second adrenal two weeks later, seek the right adrenal body and with the left index finger press slightly above and to the right of it sufficiently to permit the feeling of the pressure of this finger by the right hand placed on the back of the rabbit. With a pair of scissors, clip the hair from the back of the animal where this pressure is most distinctly felt. I recommend this because I have found that the kidneys of these animals are not always strictly in the same topographical position and the procedure suggested facilitates the location of the second incision two weeks later. After removal of all the gauze pads from the abdominal cavity, unite the muscles and peritoneum with a row of silk sutures and the skin with a second row; paint the sutures with an antiseptic collodium; dust with boric acid and apply a gauze bandage, place the animal in a warm cage and control its recovery by

visiting it twice daily. Do not feed for twenty-four hours after the operation.

## Operative Removal of a Second Adrenal in a Hare.

Inject into an animal two weeks after recovery from the first operation, 8 to 10 centigrams of morphine; then etherize. (Very little ether is necessary.) Place a pad of gauze under the abdomen and tie, belly down, upon the Harvard operating board. Clip the hair from the right flank, then shave and disinfect the skin. Make a longitudinal incision through the skin and fascia of the flank about two inches from the spinous processes of the vertebræ. Extend incision for about 3 1/2 inches downward, beginning at the inferior border of the ribs. The lumbar aponeurosis of the abdominal muscles are separated from the muscles of the spine and held apart by a retractor. As in operating through the abdomen, we must now seek for the kidney, then the renal vein and its juncture with the inferior vena cava on the right side. The right suprarenal body is found in the angle where these two vessels join. Although the operator is working on the right kidney and outside of the peritoneum, it is not always possible to remove the adrenal body entirely without injury to these vessels. Whatever is left of this body should be crushed with the forceps. Strong traction on the edges of the wound is not advisable because of likelihood of tearing the renal vessels. An assistant stands on the opposite side of the operating table and pushing the kidney down and to the right, with the left index finger he elevates the margin of the wound, upward and outward with two fingers of his right hand. After removal of the right suprarenal, the wound is sewed up and dressed as described in the preceding. This second operation should be done in the morning, for death occasionally follows within the first twenty-four hours after the removal of the second adrenal. In fact the second operation is useful only for studying the result of complete extirpation of both adrenals which closely resemble those of Addison's disease in man.

## Experiments after the Removal of the Left Adrenal Alone.

Immediately after removal of the adrenal, weigh it carefully. Grind it in a mortar with silica or quartz (aluminium silicate or kaolin is used by the author). Add 10 c.c. of normal NaCl solution during the grinding; which must continue for about half an hour, gradually adding enough salt solution to equal ten times the weight of the gland. Keep in the thermostat at 30° C. for forty-eight hours. Then press through boiled muslin and filter the liquid thus gained, through paper. The bottle in which the ground mass is placed in the thermostat must have a tightly fitting glass stopper. One c.c. of the extract finally gained approximately equals 0.1 of the fresh gland.

According to directions given in the lessons for blood-pressure experimentation, prepare a second rabbit, with a cannula in the carotid artery as well as the jugular vein. Secure both vagi on loose ligatures. Record a normal blood-pressure tracing on a kymograph. After a sufficient record has been taken, inject an amount of the suprarenal extract to equal one-tenth of the fresh gland into the jugular vein. What is the effect on rate and force of the heart beat? What is the effect on the blood pressure? How long do these effects last after the injection?

## Comparison of Adrenal Effects before and after Section of Both Vagi.

Inject enough of the extract to equal one-tenth the weight of the fresh gland. Take a blood-pressure tracing. Now cut both vagi. Take a second blood-pressure tracing. Are there any additional effects noticeable due to the elimination of the vagi?

## Study of the Rabbit after Complete Extirpation of Both Adrenal Bodies.

In man, loss of both adrenals by disease is characterized by five symptoms: 1. Great muscular weakness. 2. Nervous

prostration. 3. Loss of tone of the blood-vessels. 4. Bronze pigmentation of the skin and frequently digestive disturbances. 5. Irregularities of digestion. The latter two symptoms do not occur in animals because they do not live long enough after extirpation of these important organs to develop these symptoms, but they do show the first three groups of symptoms. Adrenalin when injected into this animal does not influence those involuntary muscles that have no sympathetic nerve supply, but feeding of desiccated adrenal bodies may keep them alive for weeks provided the animal can be made to eat this substance. Should the rabbit die, make a careful autopsy to assure yourself that the adrenal bodies had been entirely removed and also of any change that has occurred in the mucous membrane of the digestive tract.

## Biologic Test for the Presence or Absence of Epinephrin in the Blood.

*Experiments with Adrenalin.*—Disease has very aptly been compared to an experiment which is performed by nature under abnor- conditions, all experiments it is true are performed under abnormal mal conditions because all physiologic methods interfere more or less with the regular processes that go on in living things. It often becomes necessary even in the human being to ascertain whether adrenalin is present in too large or in too small amount in the blood. It is especially in Addison's disease which destroys the suprarenal capsule and in arterio-sclerosis in which high blood pressures or low blood pressures are found; the latter especially with the advanced disease of the suprarenal capsule. To test whether the blood at any given time contains too much or too little adrenalin we have to make use of some tissues whose reaction to the sympathetic nerve that supplies it, is known and suspend this tissue in the plasma to be tested and note whether the effect produced is either contraction or increase of tonus on the one hand or inhibition and loss of tonus on the other.

## Effects of Adrenalin on Various Tissues are Analogous to the Effects of Stimulating the Sympathetic Nerves that Supply those Tissues.

1. In all blood-vessels adrenalin causes constriction.

2. As we have seen in previous experiments it increases the contractile force of the heart.

3. The pupil is dilated (mydriasis).

4. The muscle of the intestine in all mammalia is inhibited with the single exception of the ileo-cecal sphincter.

5. The stomach in mammals is relaxed but in the frog it is brought to contraction so that both of these effects correspond with those obtained in stimulating the sympathetic nerve going to those tissues, in the respective species.

6. The effect of stimulation of the sympathetic nerve for the bladder and uterus varies in different animals; but no matter what this effect is, a similar one is produced by the application of adrenalin.

### Directions for Executing the Experiments.

Use the extract of the suprarenal gland made according to directions previously given, or a solution of adrenalin 1 to 1000. The tissues used for this purpose are the following: A, strips of medium-sized blood-vessel from any mammal. B. Rings of muscle cut out of rabbit's uterus. C. Strips of muscle from the intestine of a cat or rabbit. D. Enucleated eye of the frog. Whenever strips of muscles are used for these experiments they are adjusted to the muscle lever as described in the illustration accompanying the lesson on Jacques Loeb's contact irritability. (p. 83.)

EXPERIMENT 1.—Suspend a strip of femoral artery of a dog in warm dog plasma, record the events that occur. 2. Suspend it in a solution of adrenalin made in the following manner: 1 c.c. of solution of adrenalin (1 to 1000;) and 9 c.c. of normal salt solution. Immerse the muscle in the apparatus described, attach to a light muscle lever and record the contractions on a slowly moving drum. Arrange a time record in seconds.

EXPERIMENT 3.—Remove this strip from the adrenalin solution and immerse it in Ringer's solution; record the events that follow.

EXPERIMENT 4.—Remove from Ringer's solution and replace into the adrenalin solution; record the events that follow.

EXPERIMENT 5.—Make a muscle preparation from a ring of rabbit uterus cut from one of the horns of the organ; arrange as before; record the contractions on the kymograph, moving slowly. Suspend the uterine muscle first in Ringer's solution, then replace the Ringer's solution by serum gained from dog's blood. In all these experiments when the muscles are changed from an artificial solution of salts to the serum of any other mammal there will be an increase of tonus in the contraction at first; later on this tonus will diminish again, but never to a lower degree as it was in Ringer's solution. The increase of tonus produced by the mammalian serum is supposed to be due to the presence of adrenalin. Sometimes the increase in tonicity is so great on changing these muscle preparations from Ringer's solution to serum that the writing-point of the lever rises above the drum.

EXPERIMENT 5.—Repeat these experiments with a strip of longitudinal muscle excised from the intestine of a cat. Take time tracings with all of these experiments. While the effect of serum is supposed to be due to the presence of adrenalin, the phenomena observed in the experiments on Loeb's *Contact Irritability* probably have something to do with the effect.

In 1904 S. J. Metzer and Clara Meltzer Auer gave the experimental evidence that adrenalin causes dilation of the frog's pupil in very dilute solutions. While in normal frogs a subcutaneous injection or instillation of suprarenal extract has an unmistakable effect upon the pupils, such an effect can be obtained in mammals by these methods of administration only some time after the removal of the superior cervical ganglion, and even then the effect is by far not so prolonged as seen in frogs. (*Am. J. Physiol.*, Vol. XI, p. 454.)

As many tissues are innervated by antagonistic nerves that counterbalance each other reciprocally and as the sympathetic nerve supply is only one of these, adrenalin in imitating the effect

of the sympathetic can only bring about what this nerve would bring about. On the pupil both adrenalin and the sympathetic stimulate the dilator and inhibit the constrictor muscles.

The normal effect of the superior cervical ganglion is to inhibit the dilator and stimulate the constrictor muscles of the pupil. Just the reverse of what suprarenal extract would do, therefore, this substance can show its greatest effect only after the antagonistic activity of this ganglion has been removed in the mammals.

EXPERIMENT. *First Stage.*—Instill adrenalin solution into the eye of a dog. What effect is observed, if any?

*Second Stage.*—Cut the cervical sympathetic nerve in the neck; again instill adrenalin solution into the eye. Notice the effect, if any.

*Third Stage.*—Completely excise the superior cervical ganglion; the demonstrator will do this for the class and a subsequent autopsy must be made to be sure that the entire ganglion has been removed.

Now instill adrenalin; again notice the effect. How long does it last? Explain the result.

Biedl (*Innere Sekretion*) finds that in the cartilaginous fish, the Selachii, the (1) internal and (2) suprarenal bodies exist separately, but only the latter, the suprarenal, contains chromaffin cells (Balfour), and only the extract of this part contains the hemodynamic principle (adrenalin), not the inter-renal body. Biedl claims to have extirpated the inter-renal bodies in the Selachii and thereby caused their death in two to three weeks under general prostration. Selachii are the elasmobranch fishes—the sharks, skates, and dogfish.

In mammals in whom he destroyed the suprarenals leaving only a small part of the cortical part, he observed that they remained alive. But mammals in whom he left only the medullary substance, he observed that death was caused. He concludes that the cortex is the part essential for life, that which detoxicates and the medulla is the secreting part.

Borutteau and Langlais (Nagel's Handbuch, Bd. II, p. 36) have combined the detoxication hypothesis with that of internal secre-

tion. By assuming that the toxic products of metabolism of the musculature are transformed by the adrenals into the highly useful and necessary adrenalin.

## CHAPTER XIII.

### Internal Secretion of the Thyroid Gland.

As in the case of the adrenal glands, complete removal of the thyroid can only be perfectly done in two operations. At each operation one lobe of the thyroid is removed; although the student himself cannot be usually entrusted with the correct performance of this operation, it is best to permit some of the class to assist and select the more skilled members to perform thyroidectomy themselves.

### Directions for Thyroidectomy.

Select the animal about the size of a large fox terrier, have him thoroughly bathed in hot water and soap, then dry, record the weight, inject 0.12 gram morphine sulphate hypodermically. When the animal has become stupefied, disinfect the neck with bichloride of mercury solution 1 to 1000. The operation should be done with the strictest asepsis, everything used should be thoroughly sterilized before applying it to the animal. Make a median incision, carrying it as far as the thyroid cartilage and exposing the trachea, draw the edges of the wound aside with retractors, separate the neck muscles from the thyroid lobe on one side. All blood-vessels should be tied with two ligatures and cut between these. An oval reddish mass becomes apparent which is sometimes connected with the lateral lobe on the other side by an isthmus; if this isthmus is present it should be moved at the first operation. In freeing the lobe of the thyroid, learn to use the handle of the scalpel in preference to the knife. Enucleate and take out the left thyroid. The thyroid branch of the carotid artery sometimes gives difficulty from secondary hemorrhage, it should not therefore be tied too close to the carotid to avoid it slipping later on. Clean the wound with sterile salt solution, dry with sterile gauze, draw the muscles together by a row

of silk sutures, then sew the skin together by a second row of uninterrupted silk sutures, cover the wound with a layer of thin gauze after painting it with collodion, place the animal in a dog holder, do not feed for twenty-four hours, then begin feeding, giving only 8 ounces of milk at a time, have the animal visited twice daily, see that he is so fastened that he cannot reach the wound with his hind legs. If he should become fretful and noisy it is sometimes sufficient to quiet him by petting or giving a little warm milk; if this does not quiet him give 0.1 gram of morphine hypodermatically. Continue this treatment for two weeks, taking temperature daily. Repeat the operation procedure on the fifteenth day removing the remaining lobe of the thyroid. Keep a record of all symptoms after the second operation. Should the animal not show the characteristic signs of the extirpation of the thyroid, one or two things must be ascertained, *first*, whether all of the thyroid has been entirely removed, *secondly*, whether or not there are accessory thyroids present. This can only be definitely ascertained at an autopsy later on. Any small glands found at the autopsy near the region where the thyroids were taken should be hardened, sectioned, stained and examined under the microscope to ascertain whether or not they exhibit the structure of the thyroid.

*The Parathyroids.*—There are four of these bodies, two on each side, and their position varies somewhat in different animals and indeed sometimes in different individuals of the same species. The external or inferior parathyroids usually lie on the lower region of the posterior surface of the thyroid, sometimes down on the sides of the trachea. The superior or internal thyroids lie on the posterior side of the thyroid, either on the upper or the middle third of the latter. Unfortunately for this operation of thyroidectomy and parathyroidectomy the little epithelial bodies of the parathyroids are sometimes actually contained within the substance of the thyroids and it is impossible to remove the thyroids without the parathyroids. This complicates the physiological results for the experiments of Edmunds, Vassale, Generali, Biedl, Moussu, and Gley have shown that the consequences of the

first experimental extirpations of the thyroid depended upon the exclusion of two different organs, that is they are *different histologically and physiologically*. When the parathyroids alone are removed the animals quickly perish with acute symptoms, most prominent of which are tetany or muscular convulsions. When the thyroids alone are removed, the animals survive for a longer period and in that case a condition of chronic malnutrition develops which resembles the state of myxedema in man. This condition in the animal is known as *cachexia strumipriva*.

*Experiment of Thyroidectomy Continued.*—In order to study the results experimentally of removal of the thyroid, this operation should be performed on two dogs about the same time. We will call these dogs A and B. Feed dog A on milk alone for a week and dog B on beef alone. Which animal shows the gravest symptoms? It is possible that the meat-eating dog may die within a few days and the milk-eating dog live much longer. Feed dog B on a diet of bread and meat, secure a supply of fresh thyroids from the sheep in the markets and mix it with his food. A more expensive way would be to mix thyroid extract with the food. Compare the symptoms of the dog fed with the thyroids with the dog supplied with ordinary food. Compare the symptoms of thyroid removal in dog and man.

*The function of the thyroids and parathyroids are correlated in some way not yet explained*, so that the removal of one influences the function of the other.

**Parathyroidectomy.**—This is even a more difficult operation than that of thyroidectomy and had best be performed by a surgeon. Tetany is not the only consequence of the removal of these bodies. McCallum and Vogtlin (*N. Y. Med. Record*, 74, 246, 1908) have found that the parathyroids are connected with calcium metabolism and that the development of unknown toxic substances in the body is connected with this increased excretion of calcium. The symptoms of tetany and so on are prevented by giving calcium salts internally or injecting them into the veins. Similar results were obtained in human beings as the results of unintentional removal of the parathyroids. That the symptoms

of tetany are not exclusively due to the loss of calcium is suggested by the fact that bleeding of the animals suffering with tetany or infusion of salt solution into a vein causes this symptom to disappear.

## Internal Secretion of the Pancreas.

### Directions for Operative Removal of the Pancreas.

**Preparation of the Animal.**—Study the urine of a dog for three days after a diet of a weighed amount of bread and meat; test for sugar by Fehling's solution, and also ferment some of the urine in a saccharimeter. All of these tests will be negative. Now starve the dog for twelve hours, inject 0.12 grams of morphine sulphate subcutaneously, have the dog thoroughly bathed in hot water and soap, rub dry with clean towel, secure him to the dog operating board, back down. Continue the anesthesia with the A. C. E. mixture (equal parts of alcohol, chloroform and ether). Have all materials such as ligatures, salt solution and cotton pads sterilized. Shave the hair from the abdominal region in the middle line, sterilize the dog's skin with bichloride of mercury 1 to 1000. Disinfect the hands in bichloride of mercury, make an incision in the median abdominal line, draw the edges of the wound apart with the retractor. The pancreas will be seen lying between the duodenum and the skin. Separate the organ from its attachments to the omentum and mesentery and the duodenum. Tie the pancreatic duct and all arteries and veins by two ligatures and cut between. The pancreas will now be easily removable; sew the abdominal muscles together with a single row of silk sutures and the skin with the second row. Apply collodion, dust the wound with boric acid, secure the animal on broad bandages in the holder. After recovery, collect the urine every six hours and make quantitative determinations of sugar as before the operation; keep a record of the condition of the animal and the loss of weight, thirst, appetite, weakness; keep on the same amount of carbohydrates and fats as before the operation. Does complete exclusion of the carbohydrates cause disappearance of the sugar

from the urine? To what histological element in the pancreas is the control of sugar metabolism ascribed?

What is the relation of muscle glycase to the activator formed in the pancreas? Is it necessary to render the glycolytic ferment of muscle active? Are the so-called islands of Langerhans individual organs (*Sui Generis*)? Are they integral parts of the ordinary structure of the pancreas?

## Internal Secretion of the Pituitary Body.

The pituitary body is located at the base of the brain in the *sella turcica* of the sphenoid bone. Two lobes are recognizable: 1. A large anterior lobe the structure of which resembles that of the thyroid gland, and 2. a small posterior lobe known as the infundibular portion, which histologically bears some resemblance in structure to the suprarenal bodies. Anterior and posterior lobes of the *hypophysis cerebri* are connected by a stalk. The posterior lobe like the medullary portion of the suprarenal gland is of epiblastic origin. The anterior lobe is an outgrowth of the buccal cavity, whereas its nearest histologic hemologue, the thyroid, is an outgrowth of the epithelium of the pharynx. Howell first insisted on the exact study of the physiologic function in accordance with this dual histologic structure, and discovered that the hemodynamic principle was formed in the infundibular or posterior part only. Extracts of the anterior lobe were found to be inactive (*Journ. Exp. Med.*, 1898, Vol. III, p. 245).

Oliver and Schäfer, in 1895, had obtained from injections of extracts of the entire gland a rise of blood pressure together with augmentation of the force of the heart beat. Unlike extracts of the adrenal glands, the pituitary gland extracts produced these effects without slowing of the heart, but Howell working only with extracts of the infundibular portion found the pulse rate to be slowed (sometimes 40 to 60 per cent.).

Tumors of the anterior lobe of the pituitary body have been associated with acromegaly or gigantism, *i.e.*, enormous enlargement of the bones, especially of the hands, face, and extremities.

Complete removal of the pituitary body is followed by death in 24 to 48 hours (Paulesco, Harvey Cushing).[1] The secretion of the anterior lobe is the particular part that controls skeletal growth and its complete suppression rapidly results fatally. The secretion of the posterior lobe has three distinct effects: 1. On the circulation and heart causing slowing of the heart beat and rise of blood pressure produced mainly by constriction of the peripheral arterioles; 2. augmentation of the secretion of urine and increase of the size of the kidneys as measured by the oncometer; 3. increase of carbohydrate tolerance without causing alimentary glycosuria.

## Experiment with Pituitary Extract on the Rate of the Heart Beat.

Expose the heart of a large frog adjust to the heart lever or arrange it to record on the kymograph by the suspension method. Obtain a tracing of the normal heart beat over a time record in seconds. Prepare a solution of pituitary extract containing 1 mg. in 1 c.c. of normal salt solution. Inject 1 c.c. of normal salt solution (plain). Notice the effect, if any, on the heart rate. Then inject 1 c.c. of a solution of pituitary extract. How is the heart rate affected? How long does the effect last?

## Experiment Showing the Effect of Pituitary Extracts on the Blood Pressure of a Cat.

*Directions.*—Anesthetize a cat with A. C. E. mixture. Insert a cannula into the carotid artery. Insert another cannula into the jugular vein, which must be provided with a rubber tubing about 2 inches long and firmly tied to the glass cannula. Cannula and rubber tubing are filled with Ringer's solution. The rubber end must be tightly closed with a ligature and there must be no bubbles in the cannula or tubing. This cannula is used as a channel to inject filtered pituitary extracts into the jugular vein.

[1] In the human being the pituitary has been surgically removed because of disease—with actual improvement resulting.

## Preparation of Extract of Infundibular Body.

"The infundibular portion of the pituitary body is carefully separated from the hypophesis cerebri and ground to a very fine pulp. This, which constitutes about 8 per cent. of the weight of the moist gland, is extracted by acidulated water. The proteids and phosphates contained in this solution are removed by proper means and the solution concentrated and sterilized. The filtrate is practically without color and free from proteid. It represents the extract that is to be used in the physiological experiments.

## Experiment on Effect of Pituitary Extract on the Volume of the Kidney and the Secretion of Urine.

One c.c. of such an extract, when injected intravenously into a dog, causes a rise of from 15 to 45 mm. of mercury and represents 0.1 gm. of moist or 0.01 gm. of dry infundibular body. Since there is considerable inorganic matter present, the active substance in 1 c.c. must be much less than the above.

Having prepared this extract in this manner, inject 1 c.c. into the jugular vein of the cat, but not until a normal record of the blood pressure has been taken above a time record, and not until the normal blood pressure of the cat has been measured in millimeters of mercury. These records having been satisfactorily secured, then inject in the pituitary extract as directed.

What is the effect on the heart rate? What is the effect on the blood pressure? How soon does it come on after the injection of the extract? Record the number of millimeters of mercury in the manometer before and after the injection. How long does the effect last? Repeat the same dose of pituitary extract, injecting it into the jugular vein. Will the heart rate be slowed still more? Will the blood pressure rise still higher?

The kidney must be operated upon in the same manner as was already described in the chapter on the "Extirpation of the Suprarenal Gland in the Rabbit." The left kidney must be placed in the oncometer. A cannula is inserted into the ureter. A second cannula is inserted into the jugular vein and a third cannula in

the carotid, though this third cannula is not absolutely necessary to prove the main point of the inquiry.

It would be more instructive to insert a cannula into the renal artery because this shows the variations in the blood pressure of the kidney itself, more directly. The urine as it drops from the ureter can be made to record the individual drops on the kymograph by an electric arrangement. Where this is not feasible for one reason or another, the matter of determining the amount of urine secreted in a given time must be left to a student who collects the urine in an accurately graduated vessel, at the same time counting the number of drops as they flow out of the ureteral cannula in a given time.

*Effect of Pituitary Extract on the Isolated Kidney.*—Experiments can also be undertaken to determine the action of pituitary extract on the secreting cells of the isolated kidney according to the technic of Sollmann (*Amer. Jour. Physiol.*, Feb., 1908). This method is especially recommendable when the object of the inquiry is, whether the pituitary extract has a specific influence on the secreting cells of the kidney or whether the increased flow of urine observed in animals after receiving intravenous injections of pituitary extract was merely due to the increased blood pressure produced. But let us return to the experiment with the kidney in the oncometer.

Take a tracing of the volume pulse of the kidney on the kymograph from the tube connected with the oncometer; have this tube connected with a mercury manometer so that any variations in the size of the organ can be recorded in millimeters of mercury; draw a time record around the entire drum. The kidney record through the oncometer will also show the rate of the pulse. Now inject 1 c.c. of the solution containing 1 1/2 mg. of the extract through the jugular vein cannula. Mark the time on the kymograph when this extract is injected. Observe how soon after the injection the blood pressure begins to rise. If a cannula has been placed in the carotid or femoral artery at the same time, note whether the increase in size of the kidney occurs synchronously with the blood-pressure rise. A second student must confine his

attention to the counting and measuring of the drops of urine. Is the rate of the pulse slower or faster? Is the secretion of urine augmented? Express it in drops and also in cubic centimeters per minute.

## Experiment Showing the Effect of Pituitary Extract on the Size of the Blood-vessels in the Web of the Frog's Foot or in the Omentum.

Prepare a frog's hind leg as in the experiment on the direct observation of the circulation in the web of the frog's foot. Focus the web under the microscope first at a low power and at a place where there is not much pigment. The frog should be curarized or the spinal cord should be destroyed. Notice and measure with the micrometer eyepiece, the size of two small arterioles. Inject into the dorsal lymph sack, 1/2 of 1 c.c. of the solution of pituitary extract prepared according to the preceding directions. One student should observe the web through the microscope while another makes the injection. Again measure the size of the arteriole, also the rate of the movement of the corpuscles. The narrowing of the blood-vessels will be very evident. Repeat the same experiment using a solution of *adrenalin* instead of pituitary extract. In case of adrenalin the constriction is more transient (three to five minutes), while under the influence of pituitary extract it lasts longer (thirty minutes).

## Effect of Repeated Injections of Pituitary Extract on the Blood Pressure.

Insert a cannula into the carotid artery, and second into the jugular vein of a cat that has been brought under the influence of A. C. E. mixture. Arrange the carotid cannula to record the arterial pressure on a kymograph over a time record in seconds. Inject 1 c.c. of the solution of pituitary extract. Notice the rise of blood pressure. Keep on taking records of the blood pressure until it begins to fall. Repeat the injection twenty minutes after the first and a third injection forty minutes after the first and

twenty minutes after the second. It will be observed that the first injection leaves the heart and vessels in a condition of lessened irritability toward a second injection; that is, the rise of blood pressure after a second injection, if any, will not be as high as that after the first injection. This might be compared to a kind of immunity of these organs to second and third and fourth injections, which is not observed when adrenalin is injected, for after the effect of a first injection of adrenalin subsides, a second injection will be equally as effective in producing a new rise of blood pressure.

## Effect of Injection of Pituitary Extract on the Rate and Force of the Heart Beat after Both Vagi have been Cut.

If possible, use the same animal as in the preceding experiment; if not, prepare a cat or rabbit in the same manner, but isolate both vagi and secure each one with a silk thread. If the animal has been subjected to an injection of pituitary extract before, preserve the kymographic records and permit it to recover entirely from this effect, which can be recognized by the fact that the heart rate and the blood pressure return to the normal. If a fresh animal has been used, secure a record of the normal heart rate and blood pressure, raising both vagi out of the wound by the string, bring them between the blades of a sharp scissors and cut both nerves through. Now secure a tracing of the increased heart rate and the blood pressure due to section of the two vagi. Next, inject 1 c.c. of pituitary extract into the jugular vein. Record the time of the injection. Observe that the pulse rate is slow although both vagi are cut, which suggests that the pituitary extract acts directly on the vagus endings in the heart substance. What other chemical substances act in this manner?

The same effect can be produced without cutting the vagi by paralyzing the terminations of this nerve in the heart with atropine (see chapter on the Effect of Chemical Substances on the Heart). By introducing a small catheter directly into the bladder, the

effect of the injection of pituitary extract on the secretion of urine can be measured at the same time. In fact, if the only point desirable in measuring the influence of pituitary extract on urine secreted is to know the amount in a given time, it is a more physiological method, meaning less injury to the animal, to collect the urine directly from the bladder or catheterizing the ureter through the bladder in a dog.

## General Deductions from the Experiments on Blood Pressure, Heart Rate, Size of the Blood-vessels and Secretion of Urine Made after Injections of Pituitary Extract.

The student by this time should have convinced himself from experiment on the web of a frog, and also on the inflamed conjunctiva of a rabbit, that pituitary extract does narrow the caliber of the arterioles. But at the same time, the kidney oncometer experiment indicates that on the blood-vessels of the kidney it has the opposite effect, expanding them. Why one and the same chemical substance should narrow arterioles all over the body and cause their expansion in one organ only, is not satisfactorily explained. In the chapter on "Adrenalin" we have had opportunity to study similar paradoxical effects of this substance on mydriasis of the pupil which had been there explained by the research of Meltzer. Perhaps the above effects of pituitary extract will eventually be explained in a similar manner. The effects of pituitary extract which have been described in the preceding are presumed by Schaeffer to be due to hormones of which there must be at least two, a cardio-vascular hormone and a renal hormone. As a rise in blood pressure is usually accompanied by increased secretion of urine, it may be supposed that the larger urinary secretion was exclusively due to the physical assistance to the renal filtration derived from the increase of blood pressure and the blood flow within the kidneys under pituitary extract. In other words, it might have been supposed that the augmented urinary secretion was a purely mechanical result. Although this is partly true, the

pituitary extract has also a specific and exciting effect on the cells of the uriniferous tubules. This can be proved by continuing one of the experiments already outlined in the preceding, namely, we have seen that repeated doses of pituitary extract following the first injection are inactive, so far as rise of blood pressure is concerned, but although these repeated injections do not cause further rise of arterial pressure, yet marked increase in the rate of the flow of urine can still be obtained. This may be accompanied by dilatation of vessels of the kidneys but sometimes such dilatation does not occur at all, so that it is clear that neither the rise of blood pressure nor the dilatation of the vessels of the kidneys are essential to produce the diuretic effect of pituitary extract which therefore must be due to the presence of a special hormone. Polyuria has been mentioned as a symptom in cases where the pituitary body was found diseased on post-mortem examination.

## The Control of the Secretion of Adrenalin Exercised by the Sympathetic System During Emotional States.

Suspend strips of intestinal muscle of the longitudinal coat, sensitive to epinephrin, or adrenalin, 1 to 20,000,000, in the blood of a cat obtained by introducing, into the inferior vena cava to the region of the liver, a small catheter lubricated with petrolatum. The blood thus obtained should be defibrinated and applied to the intestinal strip at body temperature.

The muscle will be observed to perform rhythmic movements.

Now bring a barking dog into the presence of a second cat. Allow the dog to remain there for several minutes. Obtain the defibrinated vena cava blood of this frightened cat in the same manner as before and suspend the strip of longitudinal bowel muscle in it.

The rythmic contractions of the strip will cease. (W. G. Cannon.)

The splanchnics are the normal inhibitors of the peristaltic movements of the intestine. This is but another instance of the action of adrenalin in imitation of the sympathetic nervous system. Opium produces the same inhibition by stimulation of the splanchnic nerves.

The view that inhibition of the contracting intestinal strip is due to an increased epinephrin content is justified for the following reasons:

1. The effect was obtained in blood from the vena cava near the liver. Early in the excitation of the animal the femoral vein will not furnish blood that will have the same effect as that obtained from the vena cava near the liver.

2. Removal of the adrenal glands after tying the adrenal vessels will result in a failure of emotional excitement to produce the effect.

3. Add varying amounts of adrenalin to inactive blood. They will evoke all the degrees of relaxation that the blood from the excited cat shows at various times.

4. **"Excited"** blood which produces prompt inhibition will lose that power on standing or on being agitated with bubbling oxygen. These conditions, together with the evidence of Dreyer that splanchnic nerve stimulation increase the secretion of the adrenal glands, and that during such emotional excitement as is employed in the experiment, signs of sympathetic nerve discharges, namely, dilatation of the pupils, rapid heart and erection of the hairs of the back and tail, will be observed, prove that the inhibitory effect is due to epinephrin.

**Conference.**—Are such states and symptoms observed before and after major surgical operations in human patients?

The acute intestinal and gastric paresis (acute dilatation of the stomach) after operations on the abdominal organ, may be explained in this manner.

## Suprarenal Extract Versus Pituitary Extract.

The pituitary body occupies the sella turcica of the sphenoid bone. It may be divided into three parts, the anterior lobe, the pars intermedia and the posterior lobe. The anterior lobe is thought to form an internal secretion influencing growth. The [illegible] intermedia presents slight histologic similarities to the thyroid

[illegible]ior lobe produces a substance which has marked

physiological effects. It is thought by Herring to cause the diabetic effects observed so often in acromegaly.

Compare the effect of the injection of extract of the pituitary gland obtained from a pharmaceutic laboratory with the effect of suprarenal gland extract procured from the same source.

Intravenous injection of extracts of the pituitary body produce two well marked effects.

1. A temporary rise of blood pressure; this is not like the rise ducts adrenalin, for a second injection following the first produces no such effect, whereas the rise of pressure obtained from adrenalin may be repeated time after time. The second and following injections of pituitary extract, unless they occur at much prolonged intervals, produce only a slight fall of pressure, which is the effect produced by most tissue extracts. The rise of pressure which occurs at the first injection is, however, like that of adrenalin, produced mainly by constriction of the peripheral arterioles. Slowing of the heart may also occasionally be produced.

2. The extract has a specific effect on the kidney, and causes there, not constriction, but dilatation of the blood-vessels, which persists for a very long time. Adrenlin, on the other hand, constricts the kidney arterioles; this dilatation by pituitary extract is accompanied with pronounced diuresis. It can hardly be doubted that this is no mere accident, but that there is some definite relationship between the activity of the posterior lobe of the pituitary and the kidney function. Extracts of the anterior lobe produce neither a rise of blood pressure nor any effect upon the kidney. An extract of the infundibular portion of the pituitary gland furnished by the house of Parke, Davis and Co., gave a marked excitatory effect on strips of rabbits intestine suspended in it, the same preparation painted on the outside of the rabbits intestine produced a prompt and vigorous peristalsis, which was arrested by subsequent painting on of calcium chloride. This effect of pituitary extract is directly opposed to that of Epinephrin.

The pituitary body is essentual for life. Removal produces great depression, coma, and death in a few days.[1]

[1] But it has been successfully removed for pathologic states in man.

# CHAPTER XIV.

## IMMUNITY.

### Immunity: Theories Concerning Immunity.

In a work of this character it is almost impossible to discuss thoroughly all the facts concerned in so large a subject; for a more detailed discussion the student is advised to read the literature and text-books on this subject, especially (*Ehrlich, Gesammelte Arbeiten Zur Immunitätsforschung*, Berlin, 1904; *Immunochemie*, by Svante Arrhenius, in *Ergebnisse der Physiologie*, pp. 480 to 551, Jahrgang, VII.)

*Ehrlich's Side Chain Hypothesis.*—By the application of chemical principles, to the problems of immunity, Ehrlich developed a hypothesis concerning the nature of the action of bacterial toxins upon the cells, and of the process of antitoxin formation. According to him the action of toxins upon the cells is of a chemical nature. A toxic molecule united with the cytoplasm of a cell because some chemical group in the molecule of the toxin has a chemical affinity for some specific group in the cell protoplasm. The group in the protoplasm that combines with the toxin is termed the *receptor* while the group of the toxin that combines with the cell is termed *haptophore.* Furthermore the toxic molecule is supposed to have two groups of affinities, a *toxophore* group which may be harmful to the cell, and a *haptophore* group which has an affinity for and combines with the *receptor* of the cell. When the toxophore molecules unite to the receptors of the cell, in sufficient numbers the cell will be destroyed. It is also possible for the toxophore molecule to combine with the receptor of the cell and later be thrown off to the blood; if this takes place the cell is not destroyed. The repeated

introduction of toxic substances into the body from time to time, will stimulate the cells to an over-production of receptors, some of which are thrown off by the cell into the circulation. These receptors floating in the blood have an affinity for the toxins also, which they are capable of destroying. These free circulating receptors constitute the antitoxin, and these antitoxic groups protect the cells. Hence the serum of an immunized animal is antitoxic, because it contains free circulating receptors that can unite with the toxin. An important point is that the antitoxin for one toxin is *specific*, and will only neutralize that toxin and no other.

*Passive and Active Immunity.*—When some foreign substance or toxin is introduced into the body, under favorable conditions, it stimulates the cells, which secrete protective substances; the animal produces its own antitoxin; this form of immunity is called *active* and is the most permanent. When the serum from an animal rendered immune to a certain toxin, is injected into another animal it will also become immune against that toxin. The injected serum contains receptors that have been formed in excess, and thrown off into the blood and thereby become antitoxin. This form of immunity is called *passive.*

*Hemolysis and Cytolysis.*—The essential phenomena in hemolysis is the discharging of the hemoglobin from the stroma of the erythrocytes into the surrounding fluid. All substances that have the power to thus discharge the hemoglobin from the red cells are termed *hemolysins.* Among the agents which can cause hemolysis may be mentioned the following: Changes in the osmotic pressure of the plasma, chemical substances as the bile salts, chloroform, amyl alcohol, excess of alkali, snake venom, bacteria, certain vegetable poisons and serum of certain animals.

When distilled water is added to the corpuscles osmotic changes occur, since within the red cells are abundant salts, soluble in water, which will dialyze outward and water will osmose into the corpuscles in an effort to establish osmotic equilibrium. The water entering the red cells causes them to swell and finally rupture with escape of their hemoglobin.

Furthermore, if the corpuscles are placed in a solution of salt

more concentrated than the cells, water escapes and their cytoplasm shrinks, but no hemoglobin escapes. All the chemical agents that effect hemolysis do so by acting on the stroma of the red corpuscles. An important fact is established from the above, that during the injection of large amounts of liquids into the circulation, care should be taken not to use solutions the concentration of which is less than the blood plasma, for destruction of red corpuscles will result.

*Hemolysis by Serum.*—It is a well-known fact that the blood serum of one species of animal injected into the circulation of another animal of different species will cause hemolysis. When, for instance, a dog's blood is added to serum of a rabbit, sheep or man, the corpuscles are destroyed; in other words, hemolysis has occurred. The above is not true for the guinea-pig and rabbit. Normally, the guinea-pig's blood is not hemolytic to the red blood cells of the rabbit. The guinea-pig's blood can be made so by the injection of a few cubic centimeters of rabbit's blood into the guinea-pig's body. If no reactions set in, the injection is repeated, and in a few days the guinea-pig's serum will be hemolytic to rabbit's red corpuscles.

The hemolytic power acquired by the guinea-pig's plasma is due to two substances that must act together: these are first, the complement or alexin which is normally present in the blood; the other, the amboceptor or immune body, is acquired. The hemolytic power of the guinea-pig's blood can be destroyed when heated to 56° C. for a short time, say half hour. This hemolytic power may be restored again by the addition of fresh untreated (normal) plasma to the heated plasma. This shows that the heated plasma still contained amboceptors, but no complements, which were destroyed by the heat, and, lastly, on the addition of normal untreated blood the complements were restored.

Ehrlich believes that the amboceptor contains two side groups or affinities, one for the receptor of red cells, the other for the complement. The amboceptor is often termed the intermediate body.

*Bacteriolysis.*—If we immunize an animal against certain living

bacteria, or even the dead bacteria, and later make appropriate tests with the serum, we find the serum has the power of destroying these bacteria. In other words, the tissues have been stimulated to produce substances termed *bacteriolysins*. The bacteriolysins are specific for the special organism that was injected.

*Agglutinins and Agglutination.*—This well known phenomenon is also the result of infection with many kinds of bacteria. The serum acquires the property of clumping or agglutination of bacteria; this biologic reaction is also specific. The Widal test for the diagnosis of typhoid fever is dependent upon the above condition. All substances that cause agglutination are called *agglutinins*.

*Cytolysins* are chemic groups developed in the plasma in reaction to foreign cells, which are destroyed by them.

*Opsonins.*—In the defense of the body against pathogenic organisms the leucocytes according to Metschnikoff play an important rôle. The leucocytes, and more particularly the neutrophiles, are often called *phagocytes* because they have the power of absorbing and digesting foreign bacteria. This function is called *phagocytosis*. It is claimed by Wright and Douglas that phagocytosis depends upon the presence of certain substances in the plasma, which they call *opsonins*. Opsonins have the power of preparing the bacteria in some way, so that they are more readily assimilated by the leucocytes.

*Precipitins.*—All foreign proteins when injected into an animal may cause the appearance of precipitating substances in the serum of the animal. If we inject into an animal the serum of another animal, *e.g.*, the serum of horse into a cow, the cow's serum will form a precipitate when added to horse's blood, and furthermore it is specific only to horse's blood. Serum reactions are of importance to the chemist since they furnish a method of distinguishing between closely related proteids, and the great value of the reactions is its extreme delicacy.

From a medico-legal standpoint they offer an accurate method of determining the source of blood and serum stains. If the blood from a human is injected into a rabbit, the serum of the rabbit will develop a *specific* precipitin for human blood, and

when rabbit's serum is added to human blood it forms a precipitate, even when the blood is greatly diluted.

## Hemolytic Experiments.

EXPERIMENT 1.—Add a small amount of dog's serum to rabbit's blood, mix and let stand in a warm place for a half hour. What changes have occurred? Examine a drop under the microscope; describe what has taken place.

2. Heat 5 c.c. of the dog's serum to 56° C. for a half hour and repeat Experiment No. 1, what changes occur?

3. The serum of guinea-pig is not normally hemolytic for red cells of rabbit's blood. Add a drop of serum from a guinea-pig to a drop of rabbit's blood; place on a slide and examine under the microscope. What changes occur? Is the blood laked or not?

4. Prepare a guinea-pig as follows: inject 3 c.c. of rabbit's blood into the abdominal cavity of the guinea-pig every day until five injections have been made. After the lapse of about six days bleed the guinea-pig, allow the blood to clot, and collect the serum.

5. Add a drop of the serum from the guinea-pig to a drop of rabbit's defibrinated or centrifugalized blood, place on a slide, and examine under the microscope; what occurs?

6. Mix in a small test-tube 1 c.c. of serum with 2 c.c. of rabbit's defibrinated blood; what changes take place? Examine a small drop under the microscope; what has occurred?

7. Heat a small amount of serum to 56° C. for twenty to thirty minutes, add a small amount of this heated serum to some rabbit's blood. Are the corpuscles destroyed in this experiment?

8. To the same amount of mixture used in Experiment 7 add a small amount of fresh serum from a normal untreated guinea-pig. Does hemolysis occur, and why?

9. Place 1 or 2 c.c. each of normal blood of a dog or an ox in four test-tubes; to one add a little chloroform, to another a small amount of ether, to the third add dilute acetic acid in normal salt solution, to the fourth add 1 c.c. a 1 per cent. solution of

taurocholate or glycocholate of sodium. What changes occur in each tube?

Rapid separation of corpuscles and plasma by the centrifuge is preferable to defibrination which unavoidably breaks up many corpuscles and thus favors hemolysis.

*Immunity from Plant Toxines.*—A number of substances from the plant kingdom that act like true toxins can be injected into an animal and later the animal can be immunized against them. Of these substances may be mentioned abrin, ricin, crotin and robin. These substances resemble proteids in many respects, they have been termed *toxalbumins;* more recent research work has cast doubt on their proteid nature.

*Ehrlich's Ricin Experiments.*—Ricin is a very powerful poison which can be extracted from the seeds of the castor oil plant. It can be isolated in such purity that one one-thousandth of a milligram (0.000001 gm.) per kilo of body weight is fatal for rabbits, and solutions of 0.001 per cent. will agglutinate red corpuscles. The substance can be given either by the mouth, injected subcutaneously, or applied locally to the conjunctiva. The immunity derived from ricin is also specific.

If definitely known amounts of ricin toxin be given to an animal, in either of the three ways stated, gradually and cautiously increasing the dosage, after the lapse of from two to three weeks the animal will become immune to the substance. For white mice the dosage of 0.002 grams should be given the first day, increasing the dose until the animal can take 0.01 gram.

The fatal dose for white mice is 1 c.c. of a 1/200,000 solution per 20 grams of weight. If we inject a fatal dose into a white mouse and at the same time, as a control, inject an immunized mouse with a much larger dose it will survive, while the non-immunized animal will die. The ricin is usually given to mice in a cracker ground up with a weighed amount of toxin and fed with daily increasing amounts for sixteen to eighteen days.

*Ricin Antitoxin or Antiricin.*—It is also possible to make an antiricin or antitoxin.

Bleed an immunized mouse, defibrinate its blood, and add to

5 c.c. of this blood ricin solution in such quantity that it contains twice the fatal dose. Now inject into a normal mouse 1 c.c. of this mixture for every 20 grams of weight, and note results. Is it fatal?

By the use of *phytotoxins*, as they are termed, Ehrlich was able to determine that the toxin and antitoxin acted quantitatively; in other words, a definite amount of antitoxin neutralized a definite amount of toxin in the blood.

In addition to the hemagglutinating poison, ricin contains also a violent nerve poison. In the beginning of his work Ehrlich took these two toxins to be identical, but Bashford (*Journ. Path. and Bact.*, 8, 62, 1902) gave the evidence that they were two separate and distinct chemical bodies. A large class of poisons resemble ricin in this respect in that they contain two poisons; one acts hemolytically (especially after addition of lecithin), the second acts neurotoxically. All snake venoms and tetano-toxin belong to this class.

The existence of two such separate poisons in one and the same substance has complicated the problem of estimating exact neutralization of a poisonous substance by its antitoxin. For in the reaction that occurs when a living organism is slowly and gradually immunized, it is possible that both toxins are not equally combatted; in other words, the hemolytic portion may be neutralized but not the neurotoxin portion or *vice versa*. Madsen and Noguchi found that in neutralizing the poison of "*crotalus*" the neurotoxic portion follows a different law from that followed by the hemolytic portion. The same is true of the cobra poison. All this goes to show *that the problem of immunity is too extensive and complicated to lend itself to didactic object lessons in the physiologic laboratory*. The experiments require a highly specialized technic and broad experience of the teacher; they are also too time-robbing for the period assigned to the "*physiologic practicum*" and had more profitably be assigned to the course in bacteriology or pharmacology.

*Experiment of Danysz in Toxin Neutralization.*—This experiment was founded on the observation that when a toxin is

neutralized by an equivalent amount of its antitoxin (Arrhenius and Madsen), the toxicity of the resultant mixture is less if the entire amount of antitoxin is added *at once* than when toxin and antitoxin are neutralized in successive steps, or even only in two stages. In other words, let us assume that it requires one molecule of cholesterin to neutralize one molecule of tetanolysin (Madsen and Walbum), and furthermore that we have a solution of 10 c.c. of cholesterin solution and 10 c.c. of tetanolysin so equally balanced that 1 c.c. of cholesterin solution will exactly neutralize 1 c.c. of tetanolysin solution or 10 c.c. of the former neutralize 10 c.c. of the latter. Then if complete neutralization is desired it is best to add the 10 c.c. of cholesterin at once to the 10 c.c. of tetanolysin. The resultant mixture will not be toxic to rabbits. But if only 5 c.c. of cholesterin is added, then a pause of ten to fifteen minutes and then in a second stage the second 5 c.c. of cholesterin mixed with the tetanolysin, the mixture will not be inocuous but poisonous to rabbits.

EXPERIMENT 1.—Add 10 c.c. of a gram molecular solution of cholesterin (cholesterin $C_{27}H_{46}O = 386$) to 10 c.c. of a gram-molecular solution of saponin which according to Madsen and Noguchi has the molecular weight of 7600. Cholesterin and saponin are neutralized in the proportion of 386 to 7600. If the 10 c.c. of cholesterin solution are added all at once the resultant mixture will be inocuous to rabbits.

EXPERIMENT 2.—Add 5 c.c. of the cholesterin solution to 10 c.c. of the saponin solution. Wait thirty minutes. Now add the second portion of the cholesterin solution to the saponin solution. Allow the mixture to stand fifteen minutes. Test by injecting into a rabbit's peritoneum. The mixture is still poisonous. This experiment of Danysz (*Annales de l'Institut. Pasteur*, 16, 33, 1902), is introduced here not simply because it has a most important bearing on the pharmacology of antitoxins and the rules that govern antitoxin therapy, but because this phenomenon can be parralleled by chemically pure substances of known composition,

for instance, with monochloracetic acid, $CH_2ClCOOH$, and sodium hydroxide, NaOH.

EXPERIMENT 3.—Prepare two normal solutions, one of $CH_2ClCOOH$ and a second of NaOH. Add 2 c.c. of the monochloracetic acid solution to 2 c.c. of the sodium hydroxide solution. Test the resultant mixture with neutral red or phenolphthalein. It will be proven to be neutral at all temperatures.

EXPERIMENT 4.—Add 1 c.c. of the $CH_2ClCOOH$ acid solution to 2 c.c. of the NaOH solution. Test at intervals of ten to fifteen minutes with neutral red or phenolphthalein. A basic mixture has resulted, which can by repeated testing be proven to be gradually losing its alkalinity, especially at high temperatures. The alkalinity tends to a minimum which is reached when all of the $CH_2ClCOOH$ has been decomposed into glycolic acid and chlorhydric acid. Now add the remaining 1 c.c. of the $CH_2ClCOOH$ to this solution and test again by the same indicator. The mixture will be acid. The acidity of the mixture was caused by adding the monochloracetic acid in two portions instead of at once.

In its application to antitoxin therapy this would suggest that in the treatment of diphtheria or tetanus, etc., the complete antitoxin dose (rather a little excess than not enough) should be administered at once and not in divided portions scattered over a day.

# CHAPTER XV.

## EXPERIMENTS ON VASOMOTOR NERVES.

### Dilator and Constrictor Fibers.

EXPERIMENT 1.—Blushing rabbit's ear on stimulation. Expose the carotid and the vagus on one side. The symphathetic will be found under the vagus. Place a thread under it and cut it as low down as possible.

*Notice.—a.* The ear on the same side will become intensely red. *b.* The middle artery becomes enlarged and greatly congested. Observe which is the warmer ear and note which is the larger pupil. After the above has been done the rabbit is placed in the holder and the side which was operated on is turned uppermost.

EXPERIMENT 2.—Now tie the upper end of the sympathetic with a thread and tetanize with a moderately strong current. Turn the rabbit's ear against the light. *a.* Does it change in color? *b.* What happens to the central artery? Does it become paler or disappear altogether? *c.* Note the "latent period," between the instant of stimulation and the beginning of any change in color and notice how long a time elapses after you cease to stimulate before the ear appears normal again (or congested). *d.* Does a period of congestion follow cessation of the stimulation?

Now draw the eyelids apart, and repeat the stimulation of the symphathetic nerve. An enormous dilation of the pupil follows. Only a thin ring of the iris is visible. The latent period and the after-effects are the same as were observed in the first part of the experiment.

EXPERIMENT 3.—The effect of stimulating the chorda-tympani nerve can be studied as follows:

**Technics of the Operation.**—Narcotize a dog with morphine, tie in a holder, shave the hair from the lower jaw. Make an incision through the skin on the inner edge of the posterior half of the lower jaw about 3 cm. from the edge of the inferior maxillary. Extend the cut to within 1 cm. of the angle of the inferior maxillary. Sever the cutaneous and the anterior belly of the digastric muscles from the inferior maxillary, and draw them firmly back with a sharp hook. Now the flat and broadly fibered mylohyoid lies exposed. Cut it parallel to the edge of the inferior maxillary, and perpendicular to the course of its fibers.

A plane lies exposed, in which the ducts of Wharton and of Bartholin also a number of vessels and nerves run. The two ducts can be recognized by their bluish color and because they run sagittally. Pass two ligatures under each duct, about 4 to 6 cm. apart. Introduce olive-pointed cannulas about 4 cm. long into each duct. It is well to test the entrance by first probing into each duct with a seeker. Assure yourself that saliva runs out of the canulas. Now, with a pipette, introduce 5 c.c. of dilute acetic acid into the dog's mouth.

After a few swallowing movements the saliva runs in a stream through the cannulas. Note its consistency and test the reaction.

**Isolation and Ligation of the Secretory and of the Vasomotor Nerves of the Salivary Glands.**—This is a physiologic exercise aiming to teach in the laboratory the effects of the nerves of secretion as well as the effect of the vasomotor nerves on the salivary gland. The student should endeavor to combine in one experiment a study of these two functions as far as the salivary glands are concerned. Secretory and vasomotor effects go hand in hand. The two nerves in which we are here interested are the chorda tympani and the sympathetic (in the dog the vago-sympathetic).

**Operative Technics.**—The dissection previously completed in the dog exposes the lingual nerve about the middle of the exposed part of the lower jaw close to the bone and running to the tongue. Push aside the soft parts and the nerve can be seen centripetally located. The chorda tympani can be found

running backward to the lower jaw and in the direction of the inferior maxillary. The angle between the two nerves is a wide one.

In order to stimulate the chorda tympani successfully cut through the stem of the lingual nerve centrally from the point at which the chorda tympani is given off.

It is now evident that we are dealing with a sensory nerve when we cut the lingual. The peripheral stump in secured by a ligature and the chorda tympani is isolated for a sufficient distance to allow the application of the stimulating electrode.

It is necessary in order to avoid "current loops" to cut the lingual a second time—namely, peripherally from the point at which the chorda is given off. (Another method, requiring somewhat greater skill, is to ligate the chorda near its origin and cut it off from the lingual entirely.)

**Effects.**—After the lingual is cut, the placing of acid in the mouth no longer causes a secretion of saliva. Why? But stimulation of the chorda even with weak currents, causes an abundant secretion. Why is this?

**Stimulation of the Vagosympathetic in the Neck.**—The stimulation of the upper end causes the secretion of a tough gelatinous saliva which is so thick that it can barely flow through the canula. It is of course better to stimulate the sympathetic alone, and not jointly with the vagus. Why?

**Determination of the Vasomotor Nerve Fibers in the Chorda Tympani.**—In order to demonstrate the nerve influence on the circulation of the gland a second incision at the angle of the jaw is necessary. This will expose the jugular vein which is here divided into two branches. The inner branch takes up the blood from the parotid gland. Put a cannula in this branch close above the forking of the jugular.

If the chorda tympani is now stimulated, the gland becomes so flushed with blood that it runs from the cannula in a bright red stream showing pulsation and looking like arterial blood.

**Vasomotor Fibers Issuing from the Cord in the Anterior Roots of the Spinal Nerves.** EXPERIMENT I.—*Operative*

*technics.* Curarize a large frog, and remove the arches from the fifth, sixth, seventh, eighth, and ninth vertebræ. Spread the web of a hind foot on a microscope stage.

The vessels in the web will dilate as soon as the anterior roots are cut on one side. In order to do this, tie a silk ligature around each anterior root near its origin from the cord.

Sever each root between the ligature and the cord.

Now stimulate separately the peripheral ends of the cut nerves. Note that constriction will follow as each is stimulated and that the effect is more marked if several are stimulated at the same time.

Vasomotor nerve cells exist within the cord itself as well as in the ganglia outside of the cord.

Langley has given us a method of deciding whether the neurone cells which bring about vascular constriction are contained in the sympathetic ganglia or not. When nicotine is injected into the veins of a dog in proper doses, the passage of nerve impulses through the sympathetic ganglion cells is prevented. The same result can be had by painting the ganglia with nicotine (see p. 77).

This nicotine paralysis of the sympathetic ganglion cells can be effected in the frog and thereafter stimulation of the anterior nerve roots no longer produces any change in the blood-vessels in the web of the foot. It is, however, best to perform this nicotine experiment on a warm-blooded animal, using the lumbar nerves and observing the size of the reproductive organs. No constriction occurs when the sympathetic nerves are poisoned with nicotine. Therefore the lumbar vasomotor nerve fibers must end in connection with sympathetic nerve cells in the ganglia and a new neurone starts in these ganglia which transmits the constrictor impulses to the walls of the blood-vessels.

**Vasomotor Functions of the Spinal Cord.** EXPERIMENT 1. —Curarize a large frog. Spread the web of a foot over the special holder for this experiment. Divide the cord just posterior to the bulb. This separates the vasoconstrictor center in the medulla from the cord. Notice that there is a dilation of the blood-vessels in the web of the foot.

EXPERIMENT 2.—Stimulate the peripheral segment of the divided cord. The vessels in the foot contract. Therefore the vasomotor cells in the medulla, on the way to their respective blood-vessels pass through the spinal cord.

The dilation which immediately follows the separation of the cord from the medulla, gives place in five to ten seconds to a moderate constriction.

We interpret this by assuming that the spinal cord has taken up a vasomotor function of constriction of the medulla. The spinal cord then must contain some vasomotor cells which ordinarily are under the control of those in the medulla, but which after separation of these by severing the cord, acquire a new power of independent action.

The entire cord may be destroyed and the result is an enormous distention of the vessels in the splanchnic area, whereby the heart and great blood-vessels become almost bloodless.

This is further evidence that there are vasomotor nerve cells in the cord.

There are therefore at least three neurones in the vasomotor constrictor system.

The first has its cell body in the medulla and its axone terminates in contact with the second neuron.

The second is a spinal cell, the axone of which leaves the spinal cord and synapses in contact with a sympathetic cell or its branch.

The third is a symphathetic cell which lies apart from the cord. The neuraxone of this cell passes directly to the walls of the blood-vessel.

## Vasocontrictor Nerve Fibers in Peripheral Nerves.

The demonstration of these fibers may be made on the sciatic nerve of the frog using the dimensions of the vessels of the web of the foot, measured under the microscope as a criterion. The experiment requires three persons and some experience; above all it is necessary to avoid too much curare and too strong stimulating currents, for too much curare does not only paralyze the nerve

end plates in the muscle but the vasomotor fibers also and under stimulating currents that are too strong these fibers soon become exhausted. Though a micrometer eye-piece will enable the student to actually measure any constriction following stimulation of the peripheral end of the divided sciatic, as a rule the narrowing is ascertained only in the slowing of the blood stream during excitation.

*Directions.*—With a small amount of curare paralyze the voluntary muscles one hour before the experiment is made. Destroy the brain with a seeker. Place the frog first with abdomen up, expose right sciatic nerve on one side for a short distance and tie a ligature around it. Now turn frog back upward, gently stretch the web of the right foot over the web ring or notch with very fine pins. With a low power assure yourself that the circulation is normal. The current that is just barely susceptible to the tip of the tongue is the proper strength. Place electrodes under sciatic on the peripheral side of the ligature while a second student makes observations on the smaller vessels of the web. When the short-circuiting key is opened for a minute, the current in the web vessels slows from constriction of the small arterioles, the degree of narrowing fluctuates, first increasing a few seconds and then returning to normal.

This occurs when the intact sciatic is stimulated peripherally. Now when the *central* end of the other (left) sciatic is stimulated in the same manner, constriction of the vessels of the right web will be noticeable in well-conducted experiments. How do you explain this result?

**Demonstration of Vasodilator Fibers in the Sciatic.** Vasoconstrictor and dilator fibers react differently to 1. artificial cooling; 2. to different qualities, and rates of electric stimulation, and 3. to degeneration after section. *a.* Cool the hind leg of a cat by ice and repeat the above order of experimentation, stimulation that formerly resulted in vasoconstriction now will produce dilatation. *b.* Apply weak induction shocks at long intervals and compare them to strong ones at short intervals. The dilators answer to the latter. *c.* Four days after cutting through the sciatic nerve in

right leg of frog, stimulate the peripheral end; the vessels in web will dilate. When the fibers of any nerve are severed from their neurones (cells of origin), the fibers distal to the section degenerate. The vasoconstrictors die before the dilators, and on stimulation we now get dilatation only, because the constrictors can no longer conduct. Dilator and constrictor fibers are not always together in the same nerve trunks—the "*nervi erigentes*" and the chorda tympani nerve contain only dilator fibers.

# APPENDIX.

## ON BALANCED, PROTECTIVE AND NUTRIENT SOLUTIONS.

From the lectures on the rôle of electrolytes in the development and preservation of the living cells, the student has already become partly familiar with the great importance of the inorganic salts for living matter. Living matter contains certain salts incorporated in its protoplasm, especially potassium, sodium and calcium chloride and sodium bicarbonate. It has been found, thanks to the beautiful researches of Jacques Loeb, that most of the artificial solutions which have been known to be best suited, not only to maintain the life of marine animals, but also to sustain living tissues and even the heart of mammalia in a fairly normal condition during observation, that all of these solutions closely resemble sea water. Rogers found this to be true of the heart of the marine crab for which sea water is an excellent nutritive solution. It is remarkable that the tissues of even fresh water and land animals live longest in a solution that has the same composition as sea water. Van't Hoff's solution is the following: 100 molecules NaCl, 2.2 mol. KCl, 2 mol. $CaCl_2$, 7.8 mol. $MgCl_2$, 3.8 mol. $MgSO_4$. To this should be added a trace of $NaHCO_3$. The action of sea water becomes better if a little $CaCl_2$ is added, possibly on account of a slight antagonistic effect between Ca and Mg. Van't Hoff's solution is really artificial sea water. Loeb in a large number of experiments has demonstrated that most marine animals can live in a solution containing simply 100 mol. NaCl, 2.2 mol. KCl, 1.5 mol. $CaCl$,[2] when prepared in the right concentration (M/2). The term *balanced* solution means two conditions: 1. that the concentration of the solution shall be such that the living substance placed in it shall neither

receive nor give off any of its salts or water; in other words, the solution must be isotonic with the protoplasm. But the term has a further meaning in that it implies that the salts of the solution must balance each other, not only in an osmotic sense, but also in a pharmacologic sense, for Loeb has shown that of the three essential salts NaCl, KCl and $CaCl_2$, each one alone is poisonous to living matter, but that this toxicity can be offset by the presence of the other members in the proper proportion. Cells and marine animals can only live approximately normally in a mixture of NaCl, KCl and $CaCl_2$, in the proportion in which they exist in sea water.

That the sustaining effect of balanced solutions is not merely due to the fact that they are isotonic with the protoplasm of the living thing they happen to be supporting is proven by J. Loeb's demonstration that a marine "*Gammarus*" cannot live in a solution of cane-sugar and NaCl that is isotonic with sea water, for in such a solution it will die in one-half hour.

Even the addition of KCl and $CaCl_2$ to distilled water or to cane sugar solution does not prolong the life of these animals, for in a solution of NaCl + KCl or a solution of NaCl + $CaCl_2$ the animals also die rapidly. Only in a solution of NaCl, KCl and $CaCl_2$ in the same proportion and same concentration as these salts occur in sea water do these animals live several days. (J. Loeb, *Arch. f. d. ges. Physiol.*, 97, 394, 1903).

*Nutrient Solutions.*—The word nutrient comes from the latin "*nutrire*," to nourish, meaning, a solution that furnishes something which is capable of promoting growth or repairing waste. A nutrient solution therefore must vary in composition according to the purpose it has to fulfill, especially with regard to the question as to whether it is intended for plants or animals. One of the earliest nutrient solutions employed in biologic laboratories was that proposed by Pasteur for the yeast fungus (Torula or Saccharomyces cerevisiæ) and arrived at from an analysis of the ash of this fungus. Raulin, a pupil of Pasteur, determined which nutritive solution gave the greatest development of living matter

from a given quantity of spores of "aspergillus" and found that it possessed the following composition:

| | |
|---|---|
| Water, | 1500 g. |
| Cane sugar, | 70 g. |
| Tartaric acid. | 4 g. |
| $(NH_4)_3PO_4$, | 0.60 g. |
| $K_2CO_3$, | 0.60 g. |
| $MgCO_3$, | 0.40 g. |
| $(NH_4)_2SO_4$, | 0.25 g. |
| $ZnSO_4$, | 0.07 g. |
| $FeSO_4$, | 0.07 g. |
| $K_2SiO_3$, | 0.07 g. |

To this must be added atmospheric oxygen. The sugar, ammonia—organic acid, $SO_4$ and $PO_4$ are used for the building up of protoplasm. Whilst Ca is important for animals and higher plants it does not appear from the above list to be required for fungi.

All green plants manufacture their own living substance out of $CO_2$ of the air and the electrolytes of the soil. The salts of ammonia, the nitrates, phosphates and sulphates are made use of for the construction of nitrogenous (protein) compounds and the $CO_2$ is utilized for the formation of carbohydrates. The green plants are the laboratories in which the nutritive substances for animals and fungi are prepared. A nutritive solution for an animal must contain nitrogen in assimilable form. In recent experiments aiming to maintain the life and growth of cells of mammalia outside of the animal's body, all artificial solutions of electrolytes have been found insufficient—only the plasma of the animal itself constituted a proper nutrient and protective solution.

*Protective Solutions.*—This term is synonymous with *balanced*, solution, or at least should be so employed and the definition given under the latter term will suffice for the former. Ringer's and Locke's solutions are protective, not nutrient. I insert them both for comparison.

| | *Ringer's Sol.* | *Locke's Sol.* |
|---|---|---|
| Water | 1000 g. | 1000 g. |
| NaCl | 6 g. | 9.10 g. |
| KCl | 0.075 g. | 0.2 g. |
| $CaCl_2$ | 0.1 g. | 0.2 g. |
| $NaHCO_3$ | 0.1 g. | 0.1 g. |

To Locke's solution 1 gram of dextrose is added which makes it more effective for muscle and heart experiments.

Abderhalden found the concentration of these salts in the serum of rabbits to be 0.024 per cent. CaCl and 0.042 per cent. KCl. Indeed if we express the percentage solutions of Ringer's, Locke's and Abderhalden's figures in terms of grammolecular solutions it will be found to be approximately 100 molecules NaCl to 2 molecules of $CaCl_2$ and 2.2 molecules of KCl, which is practically the proportion in which these salts exist in sea water.

Ringer first recommended his solution for the isolated heart of cold blood animals and Locke his for warm blooded animals, which already emphasizes that the two great classes require slightly different concentrations of electrolytes to be protective. The fact that Locke's solution contains sugar might lead to the opinion that it is also a nutrient solution, but this is not so, for dextrose, although it may repair waste (as the muscle performs its work by the energy of oxidized dextrose) yet it cannot build protoplasm. Furthermore, we cannot consider the dextrose a nutrient in Locke's solution until it is proven that an isolated heart actually oxidizes it and that the sugar disappears or at least is decidedly diminished in the solution. The arguments hitherto advanced to prove this point are not convincing and from my own quantitative determinations on Locke's solution, before and after circulating it through an isolated dog heart left the impression that minute losses of sugar during the perfusion of one hour were within the limits of error.

In all balanced, protective and nutrient solutions, the presence of oxygen is absolutely necessary, in fact, for warm blooded animals it has to be supplied by bubbling it through the solutions in a pure

state, for cold-blooded and marine animals, the admixture of atmospheric air appears sufficient.

Pure NaCl does not always act as a toxic substance, and this seems puzzling to a student who reads of the necessity of KCl, NaCl and $CaCl_2$ all three together. For example, the isolated center of a Medusa *Gonionemus*, common at Woods Hole, Massachusetts, beats at once in pure NaCl, but that of *Polyorchis*, a California jelly-fish only after a long interval. (Loeb.)

This difference may be due to the fact that the cells of the center of Gonionemus have enough Calcium stored up in them in an available form from the start, whilst this is not true of Polyorchis and, therefore, in the case of the former, the beats begin immediately in a solution of pure salts because it can supply the Ca and perhaps the K from its own structure. This is not possible with Polyorchis.

As we do not know how much Na, K and Ca various tissues and organs contain, we are dealing with a feature of this problem which cannot be foretold, nor can it be estimated to what degree of readiness the tissues will part with their store of these salts or receive them. The salts of these three metals exist in the tissues in combination with colloids, viz., the proteids, carbohydrates and higher fatty acids, and they exist in a dissociated form, as ions. The salts of these three metals exert a dominating influence on all life phenomena and are especially evident in the function of the nerves, muscles and glands, in fact in all those manifestations that come under the heading of irritability, contractility, rhythmicity, automaticity, conductivity, stimulation, inhibition and secretion.

*Experiment. Effect of Electrolytes on Intestinal Peristalsis.*— Expose the intestine of a cat under anesthesia. Cover with warm sterile gauze. Apply to the outside of the intestine, a 2 per cent. solution of $BaCl_2$. Note the effect in increasing any peristalsis already present or provoking it anew from a quiescent state. Repeat the experiment with sodium citrate. Repeat the experiment with an m/8 solution of these salts. Apply them to the peritoneal surface of the intestine. When the peristalsis

has been well started, apply a solution of $CaCl_2$ or $Mg_2$. Note the arrest of the peristalsis under these salts. Repeat the experiment alternately, causing peristalsis by $BaCl_2$ and arresting it by $CaCl_2$.

*Effect of these Electrolytes on Intestinal Secretion.*—Isolate an empty loop of small intestine in an anesthetized rabbit. (Thiry-Vella method.) Drop upon the peritoneal surface of the intestine a series of drops of an m/8 solution of sodium citrate or $BaCl_2$. In ten minutes the loop begins to be filled with a clear fluid. In fifteen minutes 20 c.c. or more of intestinal juice can be collected. Empty the intestine of this fluid. Note that the secretion still continues. Wipe dry the surface of the intestine with gauze. Now apply an m/8 solution of $CaCl_2$ or $MgCl_2$. The secretion will be arrested. J. B. MacCallum has shown that the secretion of other glands can also be accelerated by the $BaCl_2$ or sodium citrate and can be arrested by $CaCl_2$ or $MgCl_2$. This is especially true of the secretion of the kidneys. In these experiments we have an evidence of the fact that intestinal peristalsis as well as secretion can be set into operation by the ions of certain electrolytes and arrested by the ions of other electrolytes. It could similarly be proven that on some mammalia a pure NaCl solution acts as a toxic. Bock and Hoffman found that solutions of NaCl and NaBr when injected into the blood cause glycosuria in the rabbit. M. H. Fischer under Jacques Loeb's direction found that the higher the concentration of NaCl, the quicker the glycosuria. (*Univ. of Cal. pub. physiol.*, Vol. I, p. 77, 1903). He also found that calcium chloride was able to counteract this effect of sodium chloride. MacCallum (*U. of Cal. pub. physiol.*, Vol. I, p. 125, 1904) discovered that in rabbits treated in this manner sugar is not only excreted through the kidneys, but through the intestines.

*Experiment. Effect of NaCl Injected into the Jugular Vein of a Rabbit on the Production of Glycosuria.*—This experiment should be executed by the demonstrator. The urine must be tested for sugar before and after the injection of NaCl. The object of the experiment, like that on the intestine, is to show the control of secretions and to a certain extent of metabolism exerted by these electrolytes.

*Experiment.*—As we have seen in our experiments on the so-called contact irritability of Jacques Loeb, the process of stimulation consists to a certain extent, at least, in the exchange of Na and K ions for Ca (perhaps Mg) ions or *vice versa* and that normal irritability depends upon the presence of these ions in definite proportion in the tissues. It is, therefore, to be expected, if this theory is correct, that a change in these proportions would alter the irritability and create conditions in the tissues that give properties they did not possess when the experiment was begun. The phenomena of contact irritability are, according to Loeb, due to the withdrawal of calcium from the muscles, or rather upon a disturbance of the normal ratio of the potassium and calcium within the muscles, because it was most readily produced by such salts as precipitate calcium or diminish the concentration of free calcium ions. (Citrates, oxalates, fluorides, carbonates, phosphates). It was also shown to occur when the muscle was brought in contact with $CO_2$, oil, glycerine, sugar and toluol. All of these effects are due to changes in the surface layer of the excised muscles. What the change consists in we do not as yet know. The following experiment demonstrates that similar changes can be produced in sensory nerve endings.

## INFLUENCING THE DEGREE OF IRRITABILITY OF SENSORY NERVES IN A DECAPITATED FROG BY SOLUTIONS OF ELECTROLYTES.

*Directions.*—Cut off the head of a frog. Suspend the trunk by a hook through the skin over the breast. Prepare a 5 per cent. solution of sulphuric acid and a 5 per cent. solution of NaOH. Place a beaker under the frog on the stand used for the Loeb's contact irritability. Fill the beaker with distilled water. Raise it so that the feet come in contact with the pure $H_2O$; they will remain in the water. Remove the water and replace it with a beaker of dilute $H_2SO_4$; raise the beaker until it touches the feet of the decapitated frog; they will be immediately withdrawn. Wash the feet with pure water. Now repeat the experiment with NaOH solution from which the foot will be promptly withdrawn. Now

comes the main point of this experiment. Place the feet again in pure water; they will not be withdrawn. Now put them for one minute in a solution of $AlCl_3$ or sodium citrate and then put them back in pure water; the feet are withdrawn immediately in a violent manner which gives the idea that the contact with the water caused most excruciating pain to the decapitated frog. This experiment is the more surprising as the contact with the pure Na citrate solution or $AlCl_3$ solution does not cause such a reaction. It is due to a hypersensitiveness of the cutaneous sensory nerves produced in a similar manner as the change in the surface layer of the muscle was produced in the experiments on contact irritability. This hypersensitiveness of the skin may be allayed by putting the feet of the frog into a normal solution of cane sugar or urea. We have now seen that any change in solutions causing a substitution of Na or K ions for Ca or *vice versa* may affect motor as well as sensory nerves, may affect the muscle directly, and also secretion. These changes when they occur in the cells themselves in muscles, nerves or glands, give rise to contraction, stimulation or secretion respectively. The normal irritability of the tissues depend upon the presence of Na, K, Ca and Mg ions in the right proportion. These are combined with colloids, proteids, carbohydrates and fatty acids, and any sudden change in the relative proportions of these ion carbohydrates, ion proteids and ion lipoids alters the properties of the tissues and gives rise to functional activity or inhibition of activity according to the sense in which the change takes place (Jacques Loeb, the "Dynamics of Living Matter," p. 95). Loeb also believes that natural rhythmic processes such as the heart beat, are due to the substitution of certain metallic ions for others and furthermore, that these substitutions are caused by enzymatic processes that go on continually. These enzymatic processes set free metallic ions from certain colloid combinations and thus render them available for others. This supposition has its analogue in the action of rennin or chymosin on milk. The milk is not coagulated by the enzyme itself, but as Loevenhart has shown, the enzyme is concerned solely in rendering available calcium, which is held naturally in combination in the

milk. As long as it is in combination the Ca is of no use for the process of coagulation, but once free it precipitates the casein (Hoppe-Seylers, *Zeitschr. f. Physiol. Chemie bd.*, 41, 1904).

These experiments demonstrate the great importance of properly balanced solutions in experimenting on various tissues.

*Mechanism of Effects of Antagonistic Salts* (Jacques Loeb).—Recent work of Loeb and Wasteneys suggests that on the surface of marine animals and even of tissues, there is probably more than one colloid, which by combination with the three salts NaCl, KCl and $CaCl_2$ maintain the degree and the kind of permeability or impermeability that is necessary for function and life. If one of the three salts is missing, the permeability of the other two is increased and then modifications in the process of life phenomena occur which have been described in many of the previous experiments. The surface lamella of cells have a definite degree of permeability or impenetrability which can be influenced in one way or other by the coaction of NaCl, KCl and $CaCl_2$. This process is compared to a form of tanning, the surface layer of tissues or cells, just as leather is tanned, by acids and alkalies. The idea is that the surface that holds the inner cell protoplasm can be made more or less penetrable or impenetrable by the ions of these three salts, and that the life of the cell depends upon a continual adaptation of the external layer to chemical changes in the surrounding medium. (Sea-water, blood, lymph). (Prokter, *Kolloidchemische Beihefte* 2, 243, 1911, also Jacques Loeb, *Biochemische Zeitschrift*, October, 1911.)

## DIRECTIONS FOR OPERATION TO FORM SECONDARY OR ACCESSORY STOMACH AND GASTRIC FISTULA.

The abdominal section is made in the lines alba beginning at the ensiform process and extending downward 8 to 9 cm.

Before the opening of the gastric cavity, it is advisable to tie off the pyloric and esophageal portion of the stomach by two sterilized thin rubber tubes.

The cut through the wall of the stomach is next only made through the peritoneum and muscularis. One has to be particularly careful that the incisions on both sides of the stomach run

approximately symmetrical to the insertion of the omentum, for only under this condition is it possible to shape the circumscribed flap into a well-formed recessus.

In the whole line of section the exterior surface of the mucosa now lies exposed, and on it the obliquely running vessels are distinctly visible in the submucosa.

These vessels are stitched under with a needle and thread and doubly ligated corresponding to the two receding wound edges.

Not until then is the mucosa cut entirely through without any hemorrhage.

Next the gastric cavity is opened up for a very small distance, and a disinfection of the inside of the stomach is made by washing it out with a 0.5 per cent. solution of HCl. What next appears as a very difficult part of the operation—namely, the cutting through of the mucosa at the basis of the flap and preparing it loose and free toward both directions—is in reality very easily executed. For the first a superficial cut is made along the entire basis of the flap (with rapidly and lightly handled scalpel). During this the assistant has to make tense the mucosa by stretching it with two forceps in order to fold out the path of the cut, for the operator.

A long gauze tampon is now laid upon the line of this incision in order to arrest the hemorrhage, then this line of incision has to be gone through once more with the knife during which the assistant again has to lift off the edges of the cut with forceps in order to make tense the submucosa, which can then be easily cut through to the muscularis. In this way one readily obtains a sufficiently broad and freely dissected flap of the mucosa on both sides of the incision. Another gauze tampon is then laid in and whilst this is gradually lifted off, the larger bleeding vessels are grasped singly and tied.

There will be four flaps of mucosa; two go to form the inner wall of the large or main stomach, one on the anterior and one on the posterior wall of the stomach, and two go to form the downward facing wall of the small or accessory stomach—one on the anterior and one on the posterior gastric wall.

Each flap of mucosa (that which belongs to the big stomach and that which belongs to the future accessory stomach) is now shaped into a vault-like recessus by sutures. The concavity of each must be directed toward the corresponding part of the stomach.

The sutures must be so inserted that they perforate the mucosa nowhere, but perforate only the peritoneum and the muscularis at the stomach wound, and only the submucosa at the flap of the mucous membrane. It is useful to mark the middle and end points of the flaps of mucosa by temporarily placed ligatures; each side of the flap of the mucosa is united with the corresponding edge of the gastric wound by four or five sutures, the line of sutures should begin at the end points of the flaps and progress toward the middle. After this the stomach wound throughout its entire extent, on the greater and lesser stomach, is closed by sutures. The fistula intended to hold the cannula is narrowed, say to about the diameter of an ordinary lead-pencil, in order to prevent a prolapsus which would otherwise inevitably occur After some practice the operation is completed in about two hours, and is borne well by the animal.

During the first period after the operation acceleration of the pulse refusal, of blood, vomiting, and sometimes paretic phenomena are noticed. These are caused reflexly by the traction upon the nerves, which run in the bridge which connect the larger with the smaller stomach. They appear more distinctly after the first more abundant feedings, and are variable in character. These unpleasant complications may be prevented either by fixation of the smaller stomach to the large one, or by fixation of the large stomach against the abdominal wall. It is advisable to make a gastric fistula, and at once inserting a permanent tube as is done in human gastrostomy. This will prevent the corroding effect of the gastric juice upon the external abdominal surface.

## AN IMPROVED OPERATIVE METHOD OF FORMING AN EXPERIMENTAL ACCESSORY (PAWLOW) STOMACH IN THE DOG.

The main object of Pawlow's operation is to secure gastric juice for physiological purposes by preparing an accessory stomach in

such a way that the secretory nerve fibers of the organ shall not be injured, that the juice can be obtained in a pure state, that is, without admixture of food, and yet the glandular apparatus be stimulated from the interior surface of the gastric mucosa as it is under normal conditions.

This operation has proved very difficult even in the hands of skilled abdominal surgeons, and when performed under perfect aseptic technics. The animals do not, as a rule, die from infection; they seem to die from the prolonged etherization. The object of the author was to devise an operation accomplishing the same purposes as that of Pawlow, and yet capable of a

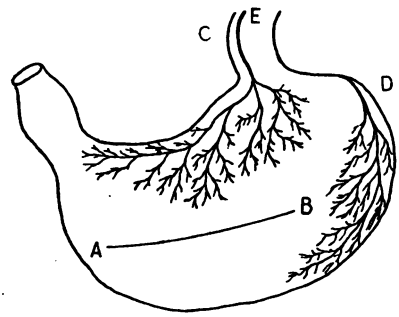



FIG. 50.—*A B*, line of Pawlow first incision. *C*, vagus and anterior gastric plexus. *D*, vagus and posterior gastric plexus. *E*, esophagus. (*J. C. Hemmeter in Am. Journ. Physiol., Vol. XVII, p.* 321.)

more rapid execution because of greater simplicity in plan. An incision is made almost along the same line as the original incision of Pawlow, but the object of this incision is not to divide the stomach into two parts, for it is only carried through the anterior wall of the stomach. (Pawlow's incision goes through the anterior and posterior wall.) The object of my incision is simply to enable the operator to push the mucosa of the stomach out through the line *A B* (Fig. 50) by invaginating the fundus or greater curvature through it. Next an incision is made only through the mucosa in a semicircular way, from the greater curvature at *C* to the greater curvature at *D*, going about as high

as the lower third of the stomach, or one-third of the distance between the greater and lesser curvature, along the line *F E G*

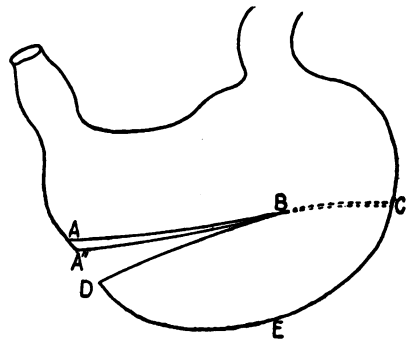



FIG. 51.—Effect and appearance after first incision according to Pawlow. The lines *AB*, *A''B*, and *DB* are closed by sutures. The accessory stomach is made out of the part enclosed by letters *BC*, *DE*. The stomach is made into two compartments by sewing together two layers of mucosa after they are dissected loose—along the dotted line *BC*. (*J. C. Hemmeter in Am. Journ. Physiol., Vol. XVII, p.* 321.)

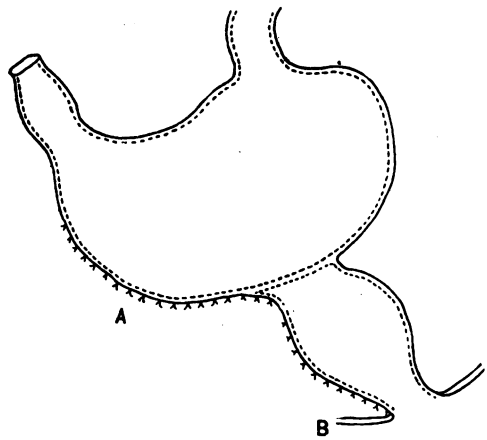



FIG. 52.—Pawlow's original operation.—*A*, Line of sutures. *B*, abdominal wall The dotted lines represent mucosa. (*J. C. Hemmeter in Am. Journ. Physiol., Vol. XVII, p.* 321.)

(Figs. 53 and 54). The incision goes through the mucosa only; the mucosa then is very slightly dissected off on either side of the incision not more than is necessary in order to catch hold of it

with the forceps, for the purpose of getting sutures through the cut ends of the mucosa. The incision is made both on the anterior and posterior walls of the stomach. As far as possible the incisions must be parallel to each other, so that when the semicir-

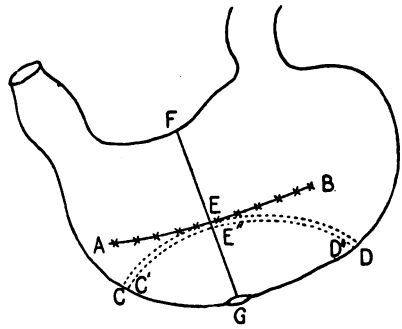



FIG. 53. (*J. C. Hemmeter in Am. Journ. Physiol., Vol. XVII, p.* 321.)

cular incision on the anterior wall is approximated to that on the posterior wall of the stomach, they coincide exactly. These two incisions are next united by silk sutures beginning at the point

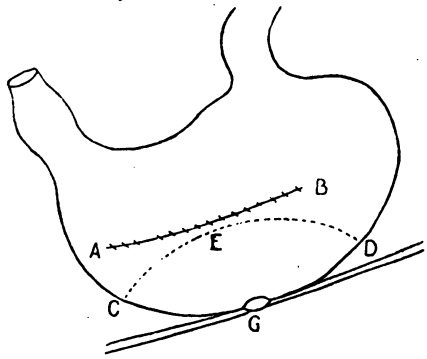



FIG. 54.—*G*, fistula on anterior abdominal wall. (*J. C. Hemmeter in Am. Journ. Physiol., Vol. XVII, p.* 321.)

*C* on the greater curvature, and making sure that the angle of the pouch is tightly walled off from the greater part of the stomach by the sutures at this point *C*. The sutures are then carried along the semicircular incision from *C* to *E* to *D*. In this way the

anterior and posterior walls are united by an inner row of silk sutures, which are inserted no deeper than the muscularis mucosæ. This has to be done by pushing the part of the greater curvature (*C E D* in Fig. 53) through the incision *A B*. When the anterior and posterior gastric walls are thus united along the lines *C E D* and *C'' E'' D''*, a circular pouch is formed (*C E D G*, in Fig. 53), separating this part of the stomach from the rest. Next the opening in the anterior gastric wall along the line *A B* is closed by sutures, a fistulous opening is made at *G*, and this point at-

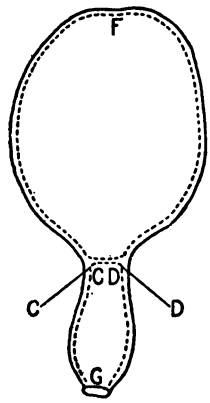



FIG. 55. (*J. C. Hemmeter in Am. Journ. Physiol., Vol. XVII, p.* 321.)

tached to the external abdominal wall as in Pawlow's method. On cross section of the stomach along the line *F G* (Fig. 53) the appearance of the main and accessory stomach when viewed from the fundus would be as represented in Fig. 55. The question might be asked, what becomes of that part of the large incision through the mucosa which faces the smaller or accessory stomach along *C* to *E* to D on the inner side of the accessory stomach, because no mention has been made that this is closed by sutures. In most of my animals I have permitted it to take care of itself, for it heals within eight to ten days, as subsequent opening of the gastric cavity at this point has proved. The secretory fibers of the vagus are not injured in this operation, which is simpler of

execution, requires less time than the Pawlow operation, and accomplishes all that this operation aims at.

A difficulty met with constantly in all animals thus operated on is the erosion and autodigestion of the skin around the abdominal opening. This untoward complication is due to the proteolytic effect of the gastric juice and to pressure by the rubber tube or cannula used to establish an outlet from the experimental accessory stomach. If the dog is permitted to lie down, there will be still larger erosions, because the abdominal integument comes to rest in a pool of escaped gastric juice.

Two things are of great assistance in this difficulty: one is the support of the animal by two broad bandages passed under the thorax anterior to the fistula and under the abdomen posterior to the same, fixing the dog to an upright holder so that he cannot lie down. The animal becomes reconciled to the holder and bandages in a few days and learns to rest and sleep in this fixture. If other animals are in the same room, the dog operated on must be blindfolded, because the secretion of gastric juice is notably influenced by psychic processes, caused by actions of the experimenter and the behavior of other animals.

Another helpful factor is the dressing of the integument around the abdominal wound during the entire time in which the animal is under actual observation, that is, during the hours when juice is collected and even during the hours of rest. The main object of this dressing is to render the gastric juice inert and at the same time to protect the surface of the skin. After testing a number of substances as dressing powders I finally settled on simple zinc oxide made alkaline with sodium bicarbonate—about one part of sodium bicarbonate to five parts of zinc oxide. During the hours of rest this powder is applied liberally all around the cannula or rubber discharge tube. But during the hours of collection of juice care must be had lest some of the alkaline powder fall into the collecting bottles and neutralize the juice. It had best be wiped off by a little absorbent cotton before the bottle is applied. While the juice is being collected it rarely spreads to the surrounding integument and the dressing is not so much needed then. It is in

the intervals between the periods of actual collection and study that the oozing of gastric juice causes the cutaneous erosion. In sewing the experimental accessory stomach to the abdominal integument, the gastric juice at times penetrates along the silk sutures into the depths of the skin. All these stitches must therefore be sealed by an alkaline collodion reapplied daily, and no experimental work undertaken until healing is complete. The manner of feeding the animal during this period requires careful study. For the first twenty-four hours nothing is given; thereafter on expiration of this period about 200 c.c. of warm milk. If the sutures are tight between the large part of the stomach and the experimental stomach, no milk should run from the cannula; if it should do so a subsequent operation to close them up may become necessary.

## STIMULATION OF HUMAN NERVES.

### Physical Electrotomes.

Whenever a current passes through a stretch of medulated nerve it spreads throughout the whole nerve—including also that part of the nerve beyond the electrodes. On applying the two electrodes of a galvanometer upon the nerve, exterior to the part stimulated, a current passes through the galvanometer because of this spreading. This spreading is a peculiar property of living nerves and can not be demonstrated after the nerve is dead. It is due to a peculiar polarization of living nerve tissues and has been called *physical electrotomes* in distinction from *physiologic electrotomes.*

Normal motor nerves in the human body follow different laws of contraction from those governing the excised nerves of a nerve-muscle preparation. When one electrode is placed on the skin over a nerve to be investigated and the other on an indifferent part of the body (back or neck)—remote from the first electrode, a *make* contraction follows the feeblest, but yet effective current when the electrode placed on the nerve is the cathode—this is the cathode *make* contraction (the *CMC* or *CCC*). A little stronger current produces anode *make* and anode *break*

contraction (or *AMC* and *ABC*), wnen the electrode on the nerve is the anode. When a very strong current is used, we get also the cathode break contraction.

These apparent deviations from the law of contraction studied in the preceeding chapter (36), is due to the nature of the spreading of the current in human tissues.

The nerve in these instances is traversed by the branching currents, both in diagonal as well as transverse directions, not merely longitudinally as in the excised nerves.

This is of great importance to the neurologist and the clinician in differentiating between a lesion of a central and a peripheral neurone. We can make use of either the faradic or the galvanic current. In such cases the electrodes cannot be applied directly to the nerve; it becomes necessary to stimulate through the overlying skin, and the "unipolar" method of stimulation is made use of. One electrode, the stimulating, is placed over the motor nerve; the other indifferent electrode is applied to some other part of the body, as the back of the neck. With the unipolar method we must remember that the smaller the electrode, the denser will be the current; for this reason the stimulating electrode is made smaller than the indifferent electrode.

When we apply electrodes on the surface of the body and the circuit is closed the current entering at the anode will diverge and pass through the body, while at the cathode the current will converge. To facilitate the passage of the current and at the same time overcome the resistance of the epidermis it is necessary to moisten the electrodes.

To interpret the results of unipolar stimulation properly it is necessary to distinguish between *physical* and *physiological electrodes*. When the stimulating electrode rests upon the skin over the nerve, the current enters the nerve at one point and leaves at another, thus there will be an anode where the current enters and a cathode where it leaves the nerve, these are termed *physiological* anodes and cathodes.

This will occur whether the stimulating electrode is the anode or the cathode, in fact it occurs at both electrodes.

By means of the unipolar method nearly all the voluntary muscles of the body can be stimulated separately. Where the motor nerve enters a muscle it is termed a "motor point" and these so-called motor points have been mapped out on the body by this form of stimulation.

When the constant current is used we may have four different contractions, two occurring at the cathode and two at the anode, both on opening and closing the current. Thus we may get cathodal closing contraction, *CCC;* cathodal opening contraction, *COC;* anodal closing contraction, *ACC;* and anodal opening con-contraction, *AOC.*

The order in which these contractions occur and the way they vary with the strength of the current is as follows:

| Weak Current. | Medium Current. | Strong Current. |
|---|---|---|
| *CCC* | *CCC* | *CCC* |
| .... | *ACC* | *ACC* |
| .... | *AOC* | *AOC* |
| .... | .... | *COC* |

With strong currents tetanus sometimes occurs both on closure and on opening of the current.

*Experiment.*—Connect eight or ten dry cells in series; place in the circuit a commutator and a simple key, using the brass electrodes; turn the pole changer so the anode is over the neck. Make and break the current; if there is no muscular contraction add more cells to the circuit until contraction occurs. Record results. Reverse the pole changer and bring the anode over the muscles, the cathode is now over the neck; make and break the current as before starting with weak current and record as before. Tabulate your results and explain them.

*The Reaction of Degeneration.*—In certain pathological conditions and whenever a nerve is cut experimentally, the portion severed from cell of origin degenerates. After a certain time the nerve loses its irritability and fails to respond to any form of stimulation. Degeneration of the nerve is accompanied by changes in the reaction to electric currents, which afford a valuable

aid in the diagnosis of the seat of the lesion in some cases of paralysis. Paralyzed muscles fail to respond to induced currents, but become hyperirritable to the galvanic current. The normal reaction is departed from and *ACC* occurs with weak currents before the *CCC*, just a reversal of the normal reaction.

To differentiate between a lesion of a central and a peripheral neurone the physician can make use of the faradic and galvanic stimulation, and physically the examination of the reflexes both in the paralyzed as well as the healthy muscles.

*Peripheral Paralysis.*—Here the reflex arc has been broken; the paralyzed muscles lose their power to respond to the faradic current. The reaction of degeneration is present; the muscles atrophy, and the reflexes are entirely absent.

*Central Paralysis.*—In this form of paralysis the lesion has occurred in a central neurone, the peripheral neurones are normal. The muscles do not degenerate, they often show a spastic condition, the reflexes are often exaggerated. The reaction of degeneration is not present. There may be symptoms of aphasia and psychical disturbances.

Electrical stimulation is also a valuable means of treatment; by stimulation the paralyzed muscles contract artificially and their nutrition is kept up, until the nerve or centers recover their power again.

# INDEX TO AUTHORS.

# GENERAL INDEX.